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(54) **AXMI-018, AXMI-020, AND AXMI-021, A FAMILY OF DELTA-ENDOTOXIN GENES AND METHODS FOR THEIR USE**

AXMI-018, AXMI-020 UND AXMI-021, DELTA-ENDOTOXIN-GENFAMILIE UND VERWENDUNGSVERFAHREN DAFÜR

AXMI-018, AXMI-020, ET AXMI-021, FAMILLE DE GENES DE DELTA-ENDOTOXINE ET LEURS METHODES D'UTILISATION

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(74) Representative: **Power, David**
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ (GB)

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(73) Proprietor: **Athenix Corporation**
Research Triangle Park, NC 27709 (US)

(72) Inventors:

- **CAROZZI, Nadine**
Raleigh, North Carolina 27615 (US)
- **HARGISS, Tracy**
Kernersville, North Carolina 27284 (US)
- **KOZIEL, Michael, G.**
Raleigh, North Carolina 27613 (US)
- **DUCK, Nicholas, B.**
Apex, North Carolina 27502 (US)

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Description

FIELD OF THE INVENTION

[0001] This invention relates to the field of molecular biology. Provided are novel genes that encode pesticidal proteins. These proteins and the nucleic acid sequences that encode them are useful in preparing pesticidal formulations and in the production of transgenic pest-resistant plants.

BACKGROUND OF THE INVENTION

[0002] *Bacillus thuringiensis* is a Gram-positive spore forming soil bacterium characterized by its ability to produce crystalline inclusions that are specifically toxic to certain orders and species of insects, but are harmless to plants and other non-targeted organisms. For this reason, compositions including *Bacillus thuringiensis* strains or their insecticidal proteins can be used as environmentally-acceptable insecticides to control agricultural insect pests or insect vectors for a variety of human or animal diseases.

[0003] Crystal (Cry) proteins (delta-endotoxins) from *Bacillus thuringiensis* have potent insecticidal activity against predominantly Lepidopteran, Dipteran, and Coleopteran larvae. These proteins also have shown activity against *Hymenoptera*, *Homoptera*, *Phthiraptera*, *Mallophaga*, and *Acari* pest orders, as well as other invertebrate orders such as *Nemathelminthes*, *Platyhelminthes*, and *Sarcomastigophora* (Feitelson (1993) "The *Bacillus Thuringiensis* family tree" in *Advanced Engineered Pesticides*, Marcel Dekker, Inc., New York, NY) These proteins were originally classified as CryI to CryV based primarily on their insecticidal activity. The major classes were *Lepidoptera*-specific (I), *Lepidoptera*- and *Diptera*-specific (II), *Coleoptera*-specific (III), *Diptera*-specific (IV), and nematode-specific (V) and (VI). The proteins were further classified into subfamilies; more highly related proteins within each family were assigned divisional letters such as Cry1A, Cry1B, Cry1C, etc. Even more closely related proteins within each division were given names such as Cry1C1, Cry1C2, etc.

[0004] A new nomenclature was recently described for the Cry genes based upon amino acid sequence homology rather than insect target specificity (Crickmore et al. (1998) *Microbiol. Mol. Biol. Rev.* 62:807-813). In the new classification, each toxin is assigned a unique name incorporating a primary rank (an Arabic number), a secondary rank (an uppercase letter), a tertiary rank (a lowercase letter), and a quaternary rank (another Arabic number). In the new classification, Roman numerals have been exchanged for Arabic numerals in the primary rank.

[0005] The crystal protein does not exhibit insecticidal activity until it has been ingested and solubilized in the insect midgut. The ingested protoxin is hydrolyzed by proteases in the insect digestive tract to an active toxic molecule. (Höfte and Whiteley (1989) *Microbiol. Rev.* 53:242-255). This toxin binds to apical brush border receptors in the midgut of the target larvae and inserts into the apical membrane creating ion channels or pores, resulting in larval death.

[0006] Delta-endotoxins generally have five conserved sequence domains, and three conserved structural domains (see, for example, de Maagd et al. (2001) *Trends Genetics* 17:193-199). The first conserved structural domain consists of seven alpha helices and is involved in membrane insertion and pore formation. Domain II consists of three beta-sheets arranged in a Greek key configuration, and domain III consists of two antiparallel beta-sheets in "jelly-roll" formation (de Maagd et al., 2001, *supra*). Domains II and III are involved in receptor recognition and binding, and are therefore considered determinants of toxin specificity.

[0007] Because of the devastation that insects can confer, there is a continual need to discover new forms of *Bacillus thuringiensis* delta-endotoxins.

BRIEF SUMMARY OF THE INVENTION

[0008] Compositions and methods for conferring pesticide resistance to bacteria, plants, plant cells, tissues and seeds are provided. Compositions include nucleic acid molecules encoding sequences for delta-endotoxin polypeptides, vectors comprising those nucleic acid molecules, and host cells comprising the vectors. Compositions also include the polypeptide sequences of the endotoxin, and antibodies to those polypeptides. The nucleotide sequences can be used in DNA constructs or expression cassettes for transformation and expression in organisms, including microorganisms and plants. The nucleotide or amino acid sequences may be synthetic sequences that have been designed for expression in an organism including, but not limited to, a microorganism or a plant. Compositions also comprise transformed bacteria, plants, plant cells, tissues, and seeds.

[0009] In particular, isolated nucleic acid molecules corresponding to delta-endotoxin nucleic acid sequences are provided. Additionally, amino acid sequences corresponding to the polynucleotides are encompassed. In particular, the present invention provides for an isolated nucleic acid molecule comprising a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 2, 4, or 6, a nucleotide sequence set forth in SEQ ID NO: 1, 3, or 5, or the delta-endotoxin nucleotide sequence deposited in a bacterial host as Accession Nos. NRRL B-30805, NRRL B-30809, and

NRRL B-30808. Nucleotide sequences that are complementary to a nucleotide sequence of the invention, or that hybridize to a sequence of the invention are also encompassed.

[0010] Methods are provided for producing the polypeptides of the invention, and for using those polypeptides for controlling or killing a lepidopteran or coleopteran pest. Methods and kits for detecting the nucleic acids and polypeptides of the invention in a sample are also included.

[0011] The compositions and methods of the invention are useful for the production of organisms with pesticide resistance, specifically bacteria and plants. These organisms and compositions derived from them are desirable for agricultural purposes. The compositions of the invention are also useful for generating altered or improved delta-endotoxin proteins that have pesticidal activity, or for detecting the presence of delta-endotoxin proteins or nucleic acids in products or organisms.

BRIEF DESCRIPTION OF FIGURES

[0012] Figure 1 shows an alignment of AXMI-018 (SEQ ID NO: 2), AXMI-020 (SEQ ID NO: 4) and AXMI-21 (SEQ ID NO: 6). Toxins having C-terminal non-toxic domains were artificially truncated as shown. AXMI-018 and AXMI-020 have the C-terminal domain common to many crystal proteins, while AXMI-021 occurs naturally truncated. The alignment shows the most highly conserved amino acid residues highlighted in black, and highly conserved amino acid residues highlighted in gray. Figures 2A and 2B show an alignment of the truncated portion of AXMI-018 with cry12Aa (SEQ ID NO:13), cry21Aa (SEQ ID NO:14), cry21Ba1 (SEQ ID NO:15), cry5Aa (SEQ ID NO:10), cry5Ab (SEQ ID NO:11), cry5Ba (SEQ ID NO:12), cry1Ac (SEQ ID NO:7), cry1Ba (SEQ ID NO:8), and cry1Ca (SEQ ID NO:9). The alignment shows the most highly conserved amino acid residues highlighted in black, and highly conserved amino acid residues highlighted in gray. Conserved group 1 in AXMI-018 is found from about amino acid residue 200 to about 222 of SEQ ID NO:2. Conserved group 2 is found from about amino acid residue 274 to about 312 of SEQ ID NO:2. Conserved group 3 is found from about amino acid residue 480 to about 533 of SEQ ID NO:2. Conserved group 4 is found from about amino acid residue 550 to about 559 of SEQ ID NO:4. Conserved group 5 is found from about amino acid residue 635 to about 644 of SEQ ID NO:2.

[0013] Figures 3A and 3B show an alignment of the truncated portion of AXMI-020 with cry12Aa (SEQ ID NO:13), cry21Aa (SEQ ID NO:14), cry21Ba1 (SEQ ID NO:15), cry5Aa (SEQ ID NO:10), cry5Ab (SEQ ID NO:11), cry5Ba (SEQ ID NO:12), cry1Ac (SEQ ID NO:7), cry1Ba (SEQ ID NO:8), and cry1Ca (SEQ ID NO:9). The alignment shows the most highly conserved amino acid residues highlighted in black, and highly conserved amino acid residues highlighted in gray. Conserved group 1 in AXMI-020 is found from about amino acid residue 200 to about 222 of SEQ ID NO:4. Conserved group 2 is found from about amino acid residue 274 to about 312 of SEQ ID NO:4. Conserved group 3 is found from about amino acid residue 480 to about 533 of SEQ ID NO:4. Conserved group 4 is found from about amino acid residue 550 to about 559 of SEQ ID NO:4. Conserved group 5 is found from about amino acid residue 635 to about 644 of SEQ ID NO:4.

[0014] Figures 4A and 4B show an alignment of the truncated portion of AXMI-021 (SEQ ID NO:6) with cry12Aa (SEQ ID NO:13), cry21Aa (SEQ ID NO:14), cry21Ba1 (SEQ ID NO:15), cry5Aa (SEQ ID NO:10), cry5Ab (SEQ ID NO:11), cry5Ba (SEQ ID NO:12), cry1Ac (SEQ ID NO:7), cry1Ba (SEQ ID NO:8), and cry1Ca (SEQ ID NO:9). Toxins having C-terminal non-toxic domains were artificially truncated as shown. The alignment shows the most highly conserved amino acid residues highlighted in black, and highly conserved amino acid residues highlighted in gray. Conserved group 1 in AXMI-021 is found from about amino acid residue 200 to about 222 of SEQ ID NO:6. Conserved group 2 is found from about amino acid residue 274 to about 316 of SEQ ID NO:6. Conserved group 3 is found from about amino acid residue 491 to about 537 of SEQ ID NO:6. Conserved group 4 is found from about amino acid residue 554 to about 564 of SEQ ID NO:6. Conserved group 5 is found from about amino acid residue 635 to about 645 of SEQ ID NO:6.

DETAILED DESCRIPTION OF THE INVENTION

[0015] The present invention is drawn to compositions and methods for regulating pest resistance in organisms, particularly plants or plant cells. The methods involve transforming organisms with a nucleotide sequence encoding a delta-endotoxin protein of the invention. In particular, the nucleotide sequences of the invention are useful for preparing plants and microorganisms that possess pesticidal activity. Thus, transformed bacteria, plants, plant cells, plant tissues and seeds are provided. Compositions are delta-endotoxin nucleic acids and proteins of *Bacillus thuringiensis*. The sequences find use in the construction of expression vectors for subsequent transformation into organisms of interest, as probes for the isolation of other delta-endotoxin genes, and for the generation of altered pesticidal proteins by methods known in the art, such as domain swapping or DNA shuffling. The proteins find use in controlling or killing lepidopteran or coleopteran pest populations and for producing compositions with pesticidal activity.

[0016] Plasmids containing the herbicide resistance nucleotide sequences of the invention were deposited in the permanent collection of the Agricultural Research Service Culture Collection, Northern Regional Research Laboratory

(NRRL) on January 13, 2005, and assigned Accession Nos. NRRL B-30805, NRRL B-30809, and NRRL B-30808, for AXMI-018, AXMI-020, and AXMI-021, respectively. The plasmid assigned Accession No. NRRL B-30805 contains an insert having nucleotides 1-3605 of AXMI-018 (SEQ ID NO:1). These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. These deposits were made merely as a convenience for those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112.

[0017] By "delta-endotoxin" is intended a toxin from *Bacillus thuringiensis* that has toxic activity against one or more pests, including, but not limited to, members of the *Lepidoptera*, *Diptera*, and *Coleoptera* orders, or a protein that has homology to such a protein. In some cases, delta-endotoxin proteins have been isolated from other organisms, including *Clostridium bifermentans* and *Paenibacillus popilliae*. Delta-endotoxin proteins include amino acid sequences deduced from the full-length nucleotide sequences disclosed herein, and amino acid sequences that are shorter than the full-length sequences, either due to the use of an alternate downstream start site, or due to processing that produces a shorter protein having pesticidal activity. Processing may occur in the organism the protein is expressed in, or in the pest after ingestion of the protein. Delta-endotoxins include proteins identified as *cry1* through *cry43*, *cyt1* and *cyt2*, and Cyt-like toxin. There are currently over 250 known species of delta-endotoxins with a wide range of specificities and toxicities. For an expansive list see Crickmore et al. (1998), Microbiol. Mol. Biol. Rev. 62:807-813, and for regular updates see Crickmore et al. (2003) "Bacillus thuringiensis toxin nomenclature," at www.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/index.

[0018] Provided herein are novel isolated nucleotide sequences that confer pesticidal activity. Also provided are the amino acid sequences of the delta-endotoxin proteins. The protein resulting from translation of this gene allows cells to control or kill pests that ingest it.

Isolated Nucleic Acid Molecules, and Variants and Fragments Thereof

[0019] One aspect of the invention pertains to isolated nucleic acid molecules comprising nucleotide sequences encoding delta-endotoxin proteins and polypeptides or biologically active portions thereof, as well as nucleic acid molecules sufficient for use as hybridization probes to identify delta-endotoxin encoding nucleic acids. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA). The nucleic acid molecule can be single-stranded or double-stranded.

[0020] An "isolated" or "purified" nucleic acid molecule or protein, or biologically active portion thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In some embodiments, an "isolated" nucleic acid is free of sequences (such as, for example, protein encoding sequences) that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For purposes of the invention, "isolated" when used to refer to nucleic acid molecules excludes isolated chromosomes. For example, in various embodiments, the isolated delta-endotoxin encoding nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. A delta-endotoxin protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of non-delta-endotoxin protein (also referred to herein as a "contaminating protein").

[0021] Nucleotide sequences encoding the proteins of the present invention include the sequence set forth in SEQ ID NO:1, 3, or 5, the delta endotoxin nucleotide sequences deposited in bacterial hosts as Accession Nos. NRRL B-30805, NRRL B-30809, and NRRL B-30808, and complements thereof. By "complement" is intended a nucleotide sequence that is sufficiently complementary to a given nucleotide sequence such that it can hybridize to the given nucleotide sequence to thereby form a stable duplex. The corresponding amino acid sequences for the delta-endotoxin proteins encoded by these nucleotide sequences are set forth in SEQ ID NO:2, 4, or 6.

[0022] Nucleic acid molecules that are fragments of these delta-endotoxin encoding nucleotide sequences are also disclosed. By "fragment" is intended a portion of the nucleotide sequence encoding a delta-endotoxin protein. A fragment of a nucleotide sequence may encode a biologically active portion of a delta-endotoxin protein, or it may be a fragment that can be used as a hybridization probe or PCR primer using methods disclosed below. Nucleic acid molecules that are fragments of a delta-endotoxin nucleotide sequence comprise at least about 50, 100, 200, 300, 400, 500, 600, 704, 800, 900, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900, 1950, 2000, 2050, 2100, 2150, 2200, 2250, 2300, 2350, 2400, 2450, 2500, 2550, 2600, 2650, 2700, 2750, 2800, 2850, 2900, 2950, 3000, 3050, 3100, 3150, 3200, 3250, 3300, 3350, 3400, 3450, 3500, 3550, 3600, 3650 contiguous nucleotides, or up to the number of nucleotides present in a full-length delta-endotoxin encoding nucleotide sequence disclosed herein (for example, 3675 nucleotides for SEQ ID NO: 1). By "contiguous" nucleotides is intended nucleotide residues that are immediately adjacent to one another. Fragments of the nucleotide sequences of the present invention will encode protein fragments that retain the biological activity of the delta-endotoxin protein and, hence, retain

pesticidal activity. By "retains activity" is intended that the fragment will have at least about 30%, at least about 50%, at least about 70%, or at least about 80% of the pesticidal activity of the delta-endotoxin protein. Methods for measuring pesticidal activity are well known in the art. See, for example, Czapla and Lang (1990) *JEcon. Entomol.* 83:2480-2485; Andrews et al. (1988) *Biochem. J.* 252:199-206; Marrone et al. (1985) *J. of Economic Entomology* 78:290-293; and U.S. Patent No. 5,743,477.

[0023] A fragment of a delta-endotoxin encoding nucleotide sequence that encodes a biologically active portion of a protein of the invention will encode at least about 15, 25, 30, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, or 1200 contiguous amino acids, or up to the total number of amino acids present in a full-length delta-endotoxin protein of the invention (for example, 1224 amino acids for SEQ ID NO: 2).

[0024] In some embodiments, delta-endotoxin proteins of the present invention are encoded by a nucleotide sequence sufficiently identical to the nucleotide sequence of SEQ ID NO: 1, 3, or 5. By "sufficiently identical" is intended an amino acid or nucleotide sequence that has at least 80% or 85% sequence identity, about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity compared to a reference sequence using one of the alignment programs described herein using standard parameters. One of skill in the art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning, and the like.

[0025] To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., percent identity = number of identical positions/total number of positions (e.g., overlapping positions) x 100). In one embodiment, the two sequences are the same length. The percent identity between two sequences can be determined using techniques similar to those described below, with or without allowing gaps. In calculating percent identity, typically exact matches are counted.

[0026] The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A nonlimiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the BLASTN and BLASTX programs of Altschul et al. (1990) *J. Mol. Biol.* 215:403. BLAST nucleotide searches can be performed with the BLASTN program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to delta-endotoxin-like nucleic acid molecules of the invention. BLAST protein searches can be performed with the BLASTX program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to delta-endotoxin protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-Blast can be used to perform an iterated search that detects distant relationships between molecules. See Altschul et al. (1997) *supra*. When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., BLASTX and BLASTN) can be used. Alignment may also be performed manually by inspection.

[0027] Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the ClustalW algorithm (Higgins et al. (1994) *Nucleic Acids Res.* 22:4673-4680). ClustalW compares sequences and aligns the entirety of the amino acid or DNA sequence, and thus can provide data about the sequence conservation of the entire amino acid sequence. The ClustalW algorithm is used in several commercially available DNA/amino acid analysis software packages, such as the ALIGNX module of the Vector NTI Program Suite (Invitrogen Corporation, Carlsbad, CA). After alignment of amino acid sequences with ClustalW, the percent amino acid identity can be assessed. A non-limiting example of a software program useful for analysis of ClustalW alignments is GeneDoc(TM). Genedoc(TM) (Karl Nicholas) allows assessment of amino acid (or DNA) similarity and identity between multiple proteins. Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0), which is part of the GCG Wisconsin Genetics Software Package, Version 10 (Accelrys, Inc., San Diego, CA). When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

[0028] Unless otherwise stated, GAP Version 10, which uses the algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48(3):443-453, will be used to determine sequence identity or similarity using the following parameters: % identity and % similarity for a nucleotide sequence using GAP Weight of 50 and Length Weight of 3, and the nws gapdna.cmp scoring matrix; % identity or % similarity for an amino acid sequence using GAP weight of 8 and length weight of 2, and the BLOSUM62 scoring program. Equivalent programs may also be used. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10. The invention also encompasses variant nucleic acid molecules selected from the group consisting of: (a) a nucleic acid molecule comprising a nucleotide sequence having at least 80% sequence identity to

the nucleotide sequence of SEQ ID NO: 1, 3, or 5, or a complement thereof, wherein said nucleotide sequence encodes a polypeptide having pesticidal activity; and (b) a nucleic acid molecule which encodes a polypeptide comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 2, 4, or 6, wherein said nucleotide sequence encodes a polypeptide having pesticidal activity. "Variants" of the delta-endotoxin encoding nucleotide sequences include those sequences that encode the delta-endotoxin proteins disclosed herein but that differ conservatively because of the degeneracy of the genetic code as well as those that are sufficiently identical as discussed above. Naturally occurring allelic variants can be identified with the use of well-known molecular biology techniques, such as polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant nucleotide sequences also include synthetically derived nucleotide sequences that have been generated, for example, by using site-directed mutagenesis but which still encode the delta-endotoxin proteins disclosed in the present invention as discussed below. Variant proteins encompassed by the present invention are biologically active, that is they continue to possess the desired biological activity of the native protein, that is, retaining pesticidal activity. By "retains activity" is intended that the variant will have at least about 30%, at least about 50%, at least about 70%, or at least about 80% of the pesticidal activity of the native protein. Methods for measuring pesticidal activity are well known in the art. See, for example, Czapla and Lang (1990) J. Econ. Entomol. 83: 2480-2485; Andrews et al, (1988) Biochem. J. 252:199-206; Marrone et al. (1985) J. of Economic Entomology 78:290-293; and U.S. Patent No. 5,743,477.

[0029] The skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of the invention thereby leading to changes in the amino acid sequence of the encoded delta-endotoxin proteins, without altering the biological activity of the proteins. Thus, variant isolated nucleic acid molecules can be created by introducing one or more nucleotide substitutions, additions, or deletions into the corresponding nucleotide sequence disclosed herein, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Such variant nucleotide sequences are also encompassed by the present invention provided they are selected from the group consisting of: (a) a nucleic acid molecule comprising a nucleotide sequence having at least 80% sequence identity to the nucleotide sequence of SEQ ID NO: 1, 3, or 5, or a complement thereof, wherein said nucleotide sequence encodes a polypeptide having pesticidal activity; and (b) a nucleic acid molecule which encodes a polypeptide comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 2, 4, or 6, wherein said nucleotide sequence encodes a polypeptide having pesticidal activity.

[0030] For example, conservative amino acid substitutions may be made at one or more predicted, nonessential amino acid residues. A "nonessential" amino acid residue is a residue that can be altered from the wild-type sequence of a delta-endotoxin protein without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[0031] Delta-endotoxins generally have five conserved sequence domains, and three conserved structural domains (see, for example, de Maagd et al. (2001) Trends Genetics 17:193-199). The first conserved structural domain consists of seven alpha helices and is involved in membrane insertion and pore formation. Domain II consists of three beta-sheets arranged in a Greek key configuration, and domain III consists of two antiparallel beta-sheets in "jelly-roll" formation (de Maagd et al., 2001, *supra*). Domains II and III are involved in receptor recognition and binding, and are therefore considered determinants of toxin specificity.

[0032] Amino acid substitutions may be made in nonconserved regions that retain function. In general, such substitutions would not be made for conserved amino acid residues, or for amino acid residues residing within a conserved motif, where such residues are essential for protein activity. Examples of residues that are conserved and that may be essential for protein activity include, for example, residues that are identical between all proteins contained in the alignments of Figures 1, 2, 3, and 4. Examples of residues that are conserved but that may allow conservative amino acid substitutions and still retain activity include, for example, residues that have only conservative substitutions between all proteins contained in the alignments of Figures 1, 2, 3, and 4. However, one of skill in the art would understand that functional variants may have minor conserved or nonconserved alterations in the conserved residues.

[0033] Alternatively, variant nucleotide sequences can be made by introducing mutations randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for the ability to confer delta-endotoxin activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly, and the activity of the protein can be determined using standard assay techniques.

[0034] Using methods such as PCR, hybridization, and the like corresponding delta-endotoxin sequences can be identified, such sequences having substantial identity to the sequences of the invention. See, for example, Sambrook

J., and Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual*. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) and Innis, et al. (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, NY).

[0035] In a hybridization method, all or part of the delta-endotoxin nucleotide sequence can be used to screen cDNA or genomic libraries. Methods for construction of such cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook and Russell, 2001, *supra*. The so-called hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ^{32}P , or any other detectable marker, such as other radioisotopes, a fluorescent compound, an enzyme, or an enzyme co-factor. Probes for hybridization can be made by labeling synthetic oligonucleotides based on the known delta-endotoxin-encoding nucleotide sequence disclosed herein. Degenerate primers designed on the basis of conserved nucleotides or amino acid residues in the nucleotide sequence or encoded amino acid sequence can additionally be used. The probe typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, about 25, at least about 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, or 400 consecutive nucleotides of delta-endotoxin encoding nucleotide sequence(s) of the invention or a fragment or variant thereof. Methods for the preparation of probes for hybridization are generally known in the art and are disclosed in Sambrook and Russell, 2001, *supra*.

[0036] For example, an entire delta-endotoxin sequence disclosed herein, or one or more portions thereof, may be used as a probe capable of specifically hybridizing to corresponding delta-endotoxin-like sequences and messenger RNAs. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique and are at least about 10 nucleotides in length, or at least about 20 nucleotides in length. Such probes may be used to amplify corresponding delta-endotoxin sequences from a chosen organism by PCR. This technique may be used to isolate additional coding sequences from a desired organism or as a diagnostic assay to determine the presence of coding sequences in an organism. Hybridization techniques include hybridization screening of plated DNA libraries (either plaques or colonies; see, for example, Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

[0037] Hybridization of such sequences may be carried out under stringent conditions. By "stringent conditions" or "stringent hybridization conditions" is intended conditions under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, or less than 500 nucleotides in length.

[0038] Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulfate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. Optionally, wash buffers may comprise about 0.1% to about 1% SDS. Duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours.

[0039] Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem.* 138:267-284: $T_m = 81.5^\circ\text{C} + 16.6 (\log M) + 0.41 (\% \text{GC}) - 0.61 (\% \text{form}) - 500/L$; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m , hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq 90\%$ identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m , those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution), the SSC concentration can be increased so that a higher temperature can be used. An

extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) Laboratory Techniques in Biochemistry and Molecular Biology— Hybridization with Nucleic Acid Probes, Part I, Chapter 2 (Elsevier, New York); and Ausubel et al., eds. (1995) Current Protocols in Molecular Biology, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

Isolated Proteins and Variants and Fragments Thereof

[0040] Delta-endotoxin proteins are also encompassed within the present invention. By "delta-endotoxin protein" is intended a protein having the amino acid sequence set forth in SEQ ID No: 2, 4, or 6. Fragments, biologically active portions, and variants thereof are also provided which have at least 80% sequence identity to the amino acid sequence of SEQ ID No: 2, 4 or 6 and which have pesticidal activity, and may be used to practice the methods of the present invention.

[0041] "Fragments" or "biologically active portions" include polypeptide fragments comprising amino acid sequences sufficiently identical to the amino acid sequence set forth in SEQ ID No: 2, 4, or 6, and that exhibit pesticidal activity. Such "fragments" or "biologically active portions" have at least 80% sequence identity to the amino acid sequence of SEQ ID No: 2, 4 or 6. Such biologically active portions can be prepared by recombinant techniques and evaluated for pesticidal activity. Methods for measuring pesticidal activity are well known in the art. See, for example, Czapla and Lang (1990) J. Econ. Entomol. 83:2480-2485; Andrews et al. (1988) Biochem. J. 252:199-206; Marrone et al. (1985) J. of Economic Entomology 78:290-293; and U.S. Patent No. 5,743,477. As used herein, a fragment comprises at least 8 contiguous amino acids of SEQ ID No: 2, 4, or 6. The invention encompasses other fragments, however, such as any fragment in the protein greater than about 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, or 1200 amino acids.

[0042] By "variants" is intended proteins or polypeptides having an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID No: 2, 4, or 6. Such variants may also be encoded by a nucleic acid molecule that hybridizes to the nucleic acid molecule of SEQ ID No: 1, 3, or 5, or a complement thereof, under stringent conditions. Such variants will retain pesticidal activity. Variants include polypeptides that differ in amino acid sequence due to mutagenesis but which have at least 80% sequence identity to the amino acid sequence of SEQ ID No: 2, 4 or 6. Variant proteins encompassed by the present invention are biologically active, that is they continue to possess the desired biological activity of the native protein, that is, retaining pesticidal activity. Methods for measuring pesticidal activity are well known in the art. See, for example, Czapla and Lang (1990) J. Econ. Entomol. 83:2480-2485; Andrews et al. (1988) Biochem. J. 252:199-206; Marrone et al. (1985) J. of Economic Entomology 78:290-293; and U.S. Patent No. 5,743,477.

[0043] Bacterial genes, such as the AXMI-018, AXMI-020, and AXMI-021 genes of this invention, quite often possess multiple methionine initiation codons in proximity to the start of the open reading frame. Often, translation initiation at one or more of these start codons will lead to generation of a functional protein. These start codons can include ATG codons. However, bacteria such as *Bacillus sp.* also recognize the codon GTG as a start codon, and proteins that initiate translation at GTG codons contain a methionine at the first amino acid. Furthermore, it is not often determined *a priori* which of these codons are used naturally in the bacterium. Thus, it is understood that use of one of the alternate methionine codons may also lead to generation of delta-endotoxin proteins that encode pesticidal activity. These delta-endotoxin proteins are encompassed in the present invention and may be used in the methods of the present invention.

[0044] Antibodies to the polypeptides of the present invention, or to variants or fragments thereof, are also encompassed. Methods for producing antibodies are well known in the art (see, for example, Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; U.S. Patent No. 4,196,265).

Altered or Improved Variants

[0045] It is recognized that DNA sequences of a delta-endotoxin may be altered by various methods, and that these alterations may result in DNA sequences encoding proteins with amino acid sequences different than that encoded by a delta-endotoxin of the present invention. This protein may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of a delta-endotoxin protein can be prepared by mutations in the DNA. This may also be accomplished by one of several forms of mutagenesis and/or in directed evolution. In some aspects, the changes encoded in the amino acid sequence will not substantially affect the function of the protein. Such variants will possess the desired pesticidal activity. However, it is understood that the ability of a delta-endotoxin to confer pesticidal activity may be improved by the use of such techniques upon the compositions of this invention. For example, one may express a delta-endotoxin in host cells that exhibit high rates of base misincorporation during DNA replication, such as XL-1 Red (Stratagene, La Jolla, CA). After propagation in such strains, one can isolate the delta-endotoxin DNA (for example by preparing plasmid DNA, or by amplifying by PCR and cloning the resulting PCR fragment into a vector), culture the delta-endotoxin

mutations in a non-mutagenic strain, and identify mutated delta-endotoxin genes with pesticidal activity, for example by performing an assay to test for pesticidal activity. Generally, the protein is mixed and used in feeding assays. See, for example Marrone et al. (1985) J. of Economic Entomology 78:290-293. Such assays can include contacting plants with one or more pests and determining the plant's ability to survive and/or cause the death of the pests. Examples of

mutations that result in increased toxicity are found in Schnepf et al. (1998) Microbiol. Mol. Biol. Rev. 62:775-806. [0046] Alternatively, alterations may be made to the protein sequence of many proteins at the amino or carboxy terminus without substantially affecting activity. These alterations can include insertions, deletions, or alterations introduced by modern molecular methods, such as PCR, including PCR amplifications that alter or extend the protein coding sequence by virtue of inclusion of amino acid encoding sequences in the oligonucleotides utilized in the PCR amplification. Alternatively, the protein sequences added can include entire protein-coding sequences, such as those used commonly in the art to generate protein fusions. Such fusion proteins are often used to (1) increase expression of a protein of interest (2) introduce a binding domain, enzymatic activity, or epitope to facilitate either protein purification, protein detection, or other experimental uses known in the art (3) target secretion or translation of a protein to a subcellular organelle, such as the periplasmic space of Gram-negative bacteria, or the endoplasmic reticulum of eukaryotic cells, the latter of which often results in glycosylation of the protein.

[0047] Variant nucleotide and amino acid sequences of the present invention also encompass sequences derived from mutagenic and recombinogenic procedures such as DNA shuffling. With such a procedure, one or more different delta-endotoxin protein coding regions can be used to create a new delta-endotoxin protein possessing the desired properties. In this manner, libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides comprising sequence regions that have substantial sequence identity and can be homologously recombined *in vitro* or *in vivo*. For example, using this approach, sequence motifs encoding a domain of interest may be shuffled between a delta-endotoxin gene of the invention and other known delta-endotoxin genes to obtain a new gene coding for a protein with an improved property of interest, such as an increased insecticidal activity. Strategies for such DNA shuffling are known in the art. See, for example, Stemmer (1994) Proc. Natl. Acad. Sci. USA 91:10747-10751; Stemmer (1994) Nature 370:389-391; Cramer et al. (1997) Nature Biotech. 15:436-438; Moore et al. (1997) J. Mol. Biol. 272:336-347; Zhang et al. (1997) Proc. Natl. Acad. Sci. USA 94:4504-4509; Cramer et al. (1998) Nature 391:288-291; and U.S. Patent Nos. 5,605,793 and 5,837,458.

[0048] Domain swapping or shuffling is another mechanism for generating altered delta-endotoxin proteins. Domains II and III may be swapped between delta-endotoxin proteins, resulting in hybrid or chimeric toxins with improved pesticidal activity or target spectrum. Methods for generating recombinant proteins and testing them for pesticidal activity are well known in the art (see, for example, Naimov et al. (2001) Appl. Environ. Microbiol. 67:5328-5330; de Maagd et al. (1996) Appl. Environ. Microbiol. 62:1537-1543; Ge et al. (1991) J. Biol. Chem. 266:17954-17958; Schnepf et al. (1990) J. Biol. Chem. 265:20923-20930; Rang et al. (1999) Appl. Environ. Microbiol. 65:2918-2925).

Vectors

[0049] A delta-endotoxin sequence of the invention may be provided in an expression cassette for expression in a plant of interest. By "plant expression cassette" is intended a DNA construct that is capable of resulting in the expression of a protein from an open reading frame in a plant cell. Typically these cassettes contain a promoter and a coding sequence. Often, such constructs will also contain a 3' untranslated region. Such constructs may contain a "signal sequence" or "leader sequence" to facilitate co-translational or post-translational transport of the peptide to certain intracellular structures such as the chloroplast (or other plastid), endoplasmic reticulum, or Golgi apparatus.

[0050] By "signal sequence" is intended a sequence that is known or suspected to result in cotranslational or post-translational peptide transport across the cell membrane. In eukaryotes, this transport typically involves secretion into the Golgi apparatus, with some resulting glycosylation. By "leader sequence" is intended any sequence that, when translated, results in an amino acid sequence sufficient to trigger co-translational transport of the peptide chain to a subcellular organelle. Thus, this includes leader sequences targeting transport and/or glycosylation by passage into the endoplasmic reticulum, passage to vacuoles, plastids including chloroplasts, mitochondria, and the like.

[0051] By "plant transformation vector" is intended a DNA molecule that is necessary for efficient transformation of a plant cell. Such a molecule may consist of one or more plant expression cassettes, and may be organized into more than one "vector" DNA molecule. For example, binary vectors are plant transformation vectors that utilize two non-contiguous DNA vectors to encode all requisite cis- and trans-acting functions for transformation of plant cells (Hellens and Mullineaux (2000) Trends in Plant Science 5:446-451). "Vector" refers to a nucleic acid construct designed for transfer between different host cells. "Expression vector" refers to a vector that has the ability to incorporate, integrate and express heterologous DNA sequences or fragments in a foreign cell. The cassette will include 5' and 3' regulatory sequences operably linked to a sequence of the invention. By "operably linked" is intended a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences

being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame. The cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes.

[0052] "Promoter" refers to a nucleic acid sequence that functions to direct transcription of a downstream coding sequence. The promoter together with other transcriptional and translational regulatory nucleic acid sequences (also termed "control sequences") are necessary for the expression of a DNA sequence of interest.

[0053] Such an expression cassette is provided with a plurality of restriction sites for insertion of the delta-endotoxin sequence to be under the transcriptional regulation of the regulatory regions.

[0054] The expression cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region (i.e., a promoter), a DNA sequence of the invention, and a translational and transcriptional termination region (i.e., termination region) functional in plants. The promoter may be native or analogous, or foreign or heterologous, to the plant host and/or to the DNA sequence of the invention. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. Where the promoter is "native" or "analogous" to the plant host, it is intended that the promoter is found in the native plant into which the promoter is introduced. Where the promoter is "foreign" or "heterologous" to the DNA sequence of the invention, it is intended that the promoter is not the native or naturally occurring promoter for the operably linked DNA sequence of the invention.

[0055] The termination region may be native with the transcriptional initiation region, may be native with the operably linked DNA sequence of interest, may be native with the plant host, or may be derived from another source (i.e., foreign or heterologous to the promoter, the DNA sequence of interest, the plant host, or any combination thereof). Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also Guerineau et al. (1991) Mol. Gen. Genet. 262:141-144; Proudfoot (1991) Cell 64:671-674; Sanfacon et al. (1991) Genes Dev. 5:141-149; Mogen et al. (1990) Plant Cell 2:1261-1272; Munroe et al. (1990) Gene 91:151-158; Ballas et al. (1989) Nucleic Acids Res. 17:7891-7903; and Joshi et al. (1987) Nucleic Acid Res. 15:9627-9639.

[0056] Where appropriate, the gene(s) may be optimized for increased expression in the transformed host cell. That is, the genes can be synthesized using host cell-preferred codons for improved expression, or may be synthesized using codons at a host-preferred codon usage frequency. Generally, the GC content of the gene will be increased. See, for example, Campbell and Gowri (1990) Plant Physiol. 92:1-11 for a discussion of host-preferred codon usage. Methods are available in the art for synthesizing plant-preferred genes. See, for example, U.S. Patent Nos. 5,380,831, and 5,436,391, and Murray et al. (1989) Nucleic Acids Res. 17:477-498.

[0057] In one embodiment, the delta-endotoxin is targeted to the chloroplast for expression. In this manner, where the delta-endotoxin is not directly inserted into the chloroplast, the expression cassette will additionally contain a nucleic acid encoding a transit peptide to direct the delta-endotoxin to the chloroplasts. Such transit peptides are known in the art. See, for example, Von Heijne et al. (1991) Plant Mol. Biol. Rep. 9:104-126; Clark et al. (1989) J. Biol. Chem. 264:17544-17550; Delta-Cioppa et al. (1987) Plant Physiol. 84:965-968; Romer et al. (1993) Biochem. Biophys. Res. Commun. 196:1414-1421; and Shah et al. (1986) Science 233:478-481.

[0058] The delta-endotoxin gene to be targeted to the chloroplast may be optimized for expression in the chloroplast to account for differences in codon usage between the plant nucleus and this organelle. In this manner, the nucleic acids of interest may be synthesized using chloroplast-preferred codons. See, for example, U.S. Patent No. 5,380,831.

Plant Transformation

[0059] Methods of the invention involve introducing a nucleotide construct into a plant. By "introducing" is intended to present to the plant the nucleotide construct in such a manner that the construct gains access to the interior of at least one cell of the plant. The methods of the invention do not require that a particular method for introducing a nucleotide construct to a plant be used, only that the nucleotide construct gains access to the interior of at least one cell of the plant. Methods for introducing nucleotide constructs into plants are known in the art including, but not limited to, stable transformation methods, transient transformation methods, and virus-mediated methods.

[0060] By "plant" is intended whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds, plant cells, propagules, embryos and progeny of the same. Plant cells can be differentiated or undifferentiated (e.g. callus, suspension culture cells, protoplasts, leaf cells, root cells, phloem cells, pollen).

[0061] "Transgenic plants" or "transformed plants" or "stably transformed" plants or cells or tissues refers to plants that have incorporated or integrated exogenous nucleic acid sequences or DNA fragments into the plant cell. These nucleic acid sequences include those that are exogenous, or not present in the untransformed plant cell, as well as those that may be endogenous, or present in the untransformed plant cell. "Heterologous" generally refers to the nucleic acid sequences that are not endogenous to the cell or part of the native genome in which they are present, and have been added to the cell by infection, transfection, microinjection, electroporation, microprojection, or the like.

[0062] Transformation of plant cells can be accomplished by one of several techniques known in the art. The delta-

endotoxin gene of the invention may be modified to obtain or enhance expression in plant cells. Typically a construct that expresses such a protein would contain a promoter to drive transcription of the gene, as well as a 3' untranslated region to allow transcription termination and polyadenylation. The organization of such constructs is well known in the art. In some instances, it may be useful to engineer the gene such that the resulting peptide is secreted, or otherwise targeted within the plant cell. For example, the gene can be engineered to contain a signal peptide to facilitate transfer of the peptide to the endoplasmic reticulum. It may also be preferable to engineer the plant expression cassette to contain an intron, such that mRNA processing of the intron is required for expression.

[0063] Typically this "plant expression cassette" will be inserted into a "plant transformation vector." This plant transformation vector may be comprised of one or more DNA vectors needed for achieving plant transformation. For example, it is a common practice in the art to utilize plant transformation vectors that are comprised of more than one contiguous DNA segment. These vectors are often referred to in the art as "binary vectors." Binary vectors as well as vectors with helper plasmids are most often used for *Agrobacterium*-mediated transformation, where the size and complexity of DNA segments needed to achieve efficient transformation is quite large, and it is advantageous to separate functions onto separate DNA molecules. Binary vectors typically contain a plasmid vector that contains the cis-acting sequences required for T-DNA transfer (such as left border and right border), a selectable marker that is engineered to be capable of expression in a plant cell, and a "gene of interest" (a gene engineered to be capable of expression in a plant cell for which generation of transgenic plants is desired). Also present on this plasmid vector are sequences required for bacterial replication. The cis-acting sequences are arranged in a fashion to allow efficient transfer into plant cells and expression therein. For example, the selectable marker gene and the delta-endotoxin are located between the left and right borders. Often a second plasmid vector contains the trans-acting factors that mediate T-DNA transfer from *Agrobacterium* to plant cells. This plasmid often contains the virulence functions (Vir genes) that allow infection of plant cells by *Agrobacterium*, and transfer of DNA by cleavage at border sequences and vir-mediated DNA transfer, as in understood in the art (Hellens and Mullineaux (2000) Trends in Plant Science 5 :446-451). Several types of *Agrobacterium* strains (e.g. LBA4404, GV3101, EHA101, EHA105, etc.) can be used for plant transformation. The second plasmid vector is not necessary for transforming the plants by other methods such as microprojection, microinjection, electroporation, polyethylene glycol, etc.

[0064] In general, plant transformation methods involve transferring heterologous DNA into target plant cells (e.g. immature or mature embryos, suspension cultures, undifferentiated callus, protoplasts, etc.), followed by applying a maximum threshold level of appropriate selection (depending on the selectable marker gene) to recover the transformed plant cells from a group of untransformed cell mass. Explants are typically transferred to a fresh supply of the same medium and cultured routinely. Subsequently, the transformed cells are differentiated into shoots after placing on regeneration medium supplemented with a maximum threshold level of selecting agent. The shoots are then transferred to a selective rooting medium for recovering rooted shoot or plantlet. The transgenic plantlet then grows into a mature plant and produces fertile seeds (e.g. Hiei et al. (1994) The Plant Journal 6:271-282; Ishida et al. (1996) Nature Biotechnology 14:745-750). Explants are typically transferred to a fresh supply of the same medium and cultured routinely. A general description of the techniques and methods for generating transgenic plants are found in Ayres and Park (1994) Critical Reviews in Plant Science 13:219-239 and Bommineni and Jauhar (1997) Maydica 42:107-120. Since the transformed material contains many cells; both transformed and non-transformed cells are present in any piece of subjected target callus or tissue or group of cells. The ability to kill non-transformed cells and allow transformed cells to proliferate results in transformed plant cultures. Often, the ability to remove non-transformed cells is a limitation to rapid recovery of transformed plant cells and successful generation of transgenic plants.

[0065] Transformation protocols as well as protocols for introducing nucleotide sequences into plants may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation. Generation of transgenic plants may be performed by one of several methods, including, but not limited to, microinjection, electroporation, direct gene transfer, introduction of heterologous DNA by *Agrobacterium* into plant cells (*Agrobacterium*-mediated transformation), bombardment of plant cells with heterologous foreign DNA adhered to particles, ballistic particle acceleration, aerosol beam transformation (U.S. Published Application No. 20010026941; U.S. Patent No. 4,945,050; International Publication No. WO 91/00915; U.S. Published Application No. 2002015066), Lec1 transformation, and various other non-particle direct-mediated methods to transfer DNA.

[0066] Methods for transformation of chloroplasts are known in the art. See, for example, Svab et al. (1990) Proc. Natl. Acad. Sci. USA 87:8526-8530; Svab and Maliga (1993) Proc. Natl. Acad. Sci. USA 90:913-917; Svab and Maliga (1993) EMBO J. 12:601-606. The method relies on particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination. Additionally, plastid transformation can be accomplished by transactivation of a silent plastid-borne transgene by tissue-preferred expression of a nuclear-encoded and plastid-directed RNA polymerase. Such a system has been reported in McBride et al. (1994) Proc. Natl. Acad. Sci. USA 91:7301-7305.

[0067] Following integration of heterologous foreign DNA into plant cells, one then applies a maximum threshold level of appropriate selection in the medium to kill the untransformed cells and separate and proliferate the putatively trans-

formed cells that survive from this selection treatment by transferring regularly to a fresh medium. By continuous passage and challenge with appropriate selection, one identifies and proliferates the cells that are transformed with the plasmid vector. Molecular and biochemical methods can then be used to confirm the presence of the integrated heterologous gene(s) of interest into the genome of the transgenic plant.

[0068] The cells that have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick et al. (1986) Plant Cell Reports 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid having constitutive expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure expression of the desired phenotypic characteristic has been achieved. In this manner, the present invention provides transformed seed (also referred to as "transgenic seed") having a nucleotide construct of the invention, for example, an expression cassette of the invention, stably incorporated into their genome.

Evaluation of Plant Transformation

[0069] Following introduction of heterologous foreign DNA into plant cells, the transformation or integration of the heterologous gene(s) in the plant genome is confirmed by various methods such as analysis of nucleic acids, proteins and metabolites associated with the integrated gene.

[0070] PCR analysis is a rapid method to screen transformed cells, tissue or shoots for the presence of incorporated gene(s) at the earlier stage before transplanting into the soil (Sambrook and Russell (2001) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). PCR is carried out using oligonucleotide primers specific to the gene of interest or *Agrobacterium* vector background, etc.

[0071] Plant transformation may be confirmed by Southern blot analysis of genomic DNA (Sambrook and Russell, 2001, *supra*). In general, total DNA is extracted from the transformant, digested with appropriate restriction enzymes, fractionated in an agarose gel and transferred to a nitrocellulose or nylon membrane. The membrane or "blot" is subsequently probed with, for example, radiolabeled ³²P target DNA fragment to confirm the integration of introduced gene in the plant genome according to standard techniques (Sambrook and Russell, 2001, *supra*).

[0072] In Northern blot analysis, RNA is isolated from specific tissues of transformant, fractionated in a formaldehyde agarose gel, and blotted onto a nylon filter according to standard procedures that are routinely used in the art (Sambrook and Russell, 2001, *supra*). Expression of RNA encoded by the delta-endotoxin is then tested by hybridizing the filter to a radioactive probe derived from a delta-endotoxin, by methods known in the art (Sambrook and Russell, 2001, *supra*).

[0073] Western blot and biochemical assays and the like may be carried out on the transgenic plants to confirm the presence of protein encoded by the delta-endotoxin gene by standard procedures (Sambrook and Russell, 2001, *supra*) using antibodies that bind to one or more epitopes present on the delta-endotoxin protein.

Pesticidal Activity in Plants

[0074] In another aspect of the invention, one may generate transgenic plants expressing a delta-endotoxin that has pesticidal activity. Methods described above by way of example may be utilized to generate transgenic plants, but the manner in which the transgenic plant cells are generated is not critical to this invention. Methods known or described in the art such as *Agrobacterium*-mediated transformation, biolistic transformation, and non-particle-mediated methods may be used at the discretion of the experimenter. Plants expressing a delta-endotoxin may be isolated by common methods described in the art, for example by transformation of callus, selection of transformed callus, and regeneration of fertile plants from such transgenic callus. In such process, one may use any gene as a selectable marker so long as its expression in plant cells confers ability to identify or select for transformed cells.

[0075] A number of markers have been developed for use with plant cells, such as resistance to chloramphenicol, the aminoglycoside G418, hygromycin, or the like. Other genes that encode a product involved in metabolism may also be used as selectable markers. For example, genes that provide resistance to plant herbicides such as glyphosate, bromoxynil, or imidazolinone may find particular use. Such genes have been reported (Stalker et al. (1985) J. Biol. Chem. 263:6310-6314 (bromoxynil resistance nitrilase gene); and Sathasivan et al. (1990) Nucl. Acids Res. 18:2188 (AHAS imidazolinone resistance gene)).

[0076] Fertile plants expressing a delta-endotoxin may be tested for pesticidal activity, and the plants showing optimal activity selected for further breeding. Methods are available in the art to assay for pest activity. Generally, the protein is mixed and used in feeding assays. See, for example Marrone et al. (1985) J. of Economic Entomology 78:290-293.

[0077] The present invention may be used for transformation of any plant species, including, but not limited to, monocots and dicots. Examples of plants of interest include, but are not limited to, corn (maize), sorghum, wheat, sunflower, tomato, crucifers, peppers, potato, cotton, rice, soybean, sugarbeet, sugarcane, tobacco, barley, and oilseed rape, *Brassica* sp., alfalfa, rye, millet, safflower, peanuts, sweet potato, cassava, coffee, coconut, pineapple, citrus trees, cocoa, tea, banana,

avocado, fig, guava, mango, olive, papaya, cashew, macadamia, almond, oats, vegetables, ornamentals, and conifers.

[0078] Vegetables include, but are not limited to, tomatoes, lettuce, green beans, lima beans, peas, and members of the genus *Curcumis* such as cucumber, cantaloupe, and musk melon. Ornamentals include, but are not limited to, azalea, hydrangea, hibiscus, roses, tulips, daffodils, petunias, carnation, poinsettia, and chrysanthemum. In some embodiments, plants of the present invention are crop plants (for example, maize, sorghum, wheat, sunflower, tomato, crucifers, peppers, potato, cotton, rice, soybean, sugarbeet, sugarcane, tobacco, barley, oilseed rape., etc.).

Use in Pesticidal Control

[0079] General methods for employing strains comprising a nucleotide sequence of the present invention, or a variant thereof, in pesticide control or in engineering other organisms as pesticidal agents are known in the art. See, for example U.S. Patent No. 5,039,523 and EP 0480762A2.

[0080] The *Bacillus* strains containing a nucleotide sequence of the present invention, or a variant thereof, or the microorganisms that have been genetically altered to contain a pesticidal gene and protein may be used for protecting agricultural crops and products from pests. In one aspect of the invention, whole, i.e., unlysed, cells of a toxin (i.e., pesticide)-producing organism are treated with reagents that prolong the activity of the toxin produced in the cell when the cell is applied to the environment of target pest(s).

[0081] Alternatively, the pesticide is produced by introducing a delta-endotoxin gene into a cellular host. Expression of the delta-endotoxin gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. In one aspect of this invention, these cells are then treated under conditions that prolong the activity of the toxin produced in the cell when the cell is applied to the environment of target pest(s). The resulting product retains the toxicity of the toxin. These naturally encapsulated pesticides may then be formulated in accordance with conventional techniques for application to the environment hosting a target pest, e.g., soil, water, and foliage of plants. See, for example EPA 0192319, and the references cited therein. Alternatively, one may formulate the cells expressing a gene of this invention such as to allow application of the resulting material as a pesticide.

[0082] The active ingredients of the present invention are normally applied in the form of compositions and can be applied to the crop area or plant to be treated, simultaneously or in succession, with other compounds. These compounds can be fertilizers, weed killers, cryoprotectants, surfactants, detergents, pesticidal soaps, dormant oils, polymers, and/or time-release or biodegradable carrier formulations that permit long-term dosing of a target area following a single application of the formulation. They can also be selective herbicides, chemical insecticides, virucides, microbicides, amoebicides, pesticides, fungicides, bacteriocides, nematocides, molluscides or mixtures of several of these preparations, if desired, together with further agriculturally acceptable carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation. Suitable carriers and adjuvants can be solid or liquid and correspond to the substances ordinarily employed in formulation technology, e.g. natural or regenerated mineral substances, solvents, dispersants, wetting agents, tackifiers, binders or fertilizers. Likewise the formulations may be prepared into edible "baits" or fashioned into pest "traps" to permit feeding or ingestion by a target pest of the pesticidal formulation.

[0083] Methods of applying an active ingredient of the present invention or an agrochemical composition of the present invention that contains at least one of the pesticidal proteins produced by the bacterial strains of the present invention include leaf application, seed coating and soil application. The number of applications and the rate of application depend on the intensity of infestation by the corresponding pest.

[0084] The composition may be formulated as a powder, dust, pellet, granule, spray, emulsion, colloid, solution, or such like, and may be prepared by such conventional means as desiccation, lyophilization, homogenation, extraction, filtration, centrifugation, sedimentation, or concentration of a culture of cells comprising the polypeptide. In all such compositions that contain at least one such pesticidal polypeptide, the polypeptide may be present in a concentration of from about 1% to about 99% by weight.

[0085] Lepidopteran or coleopteran pests may be killed or reduced in numbers in a given area by the methods of the invention, or may be prophylactically applied to an environmental area to prevent infestation by a susceptible pest. In some embodiments, the pest ingests, or is contacted with, a pesticidally-effective amount of the polypeptide. By "pesticidally-effective amount" is intended an amount of the pesticide that is able to bring about death to at least one pest, or to noticeably reduce pest growth, feeding, or normal physiological development. This amount will vary depending on such factors as, for example, the specific target pests to be controlled, the specific environment, location, plant, crop, or agricultural site to be treated, the environmental conditions, and the method, rate, concentration, stability, and quantity of application of the pesticidally-effective polypeptide composition. The formulations may also vary with respect to climatic conditions, environmental considerations, and/or frequency of application and/or severity of pest infestation.

[0086] The pesticide compositions described may be made by formulating either the bacterial cell, crystal and/or spore suspension, or isolated protein component with the desired agriculturally-acceptable carrier. The compositions may be formulated prior to administration in an appropriate means such as lyophilized, freeze-dried, desiccated, or in an aqueous carrier, medium or suitable diluent, such as saline or other buffer. The formulated compositions may be in the form of a

dust or granular material, or a suspension in oil (vegetable or mineral), or water or oil/water emulsions, or as a wettable powder, or in combination with any other carrier material suitable for agricultural application. Suitable agricultural carriers can be solid or liquid and are well known in the art. The term "agriculturally-acceptable carrier" covers all adjuvants, inert components, dispersants, surfactants, tackifiers, binders, etc. that are ordinarily used in pesticide formulation technology; these are well known to those skilled in pesticide formulation. The formulations may be mixed with one or more solid or liquid adjuvants and prepared by various means, e.g., by homogeneously mixing, blending and/or grinding the pesticidal composition with suitable adjuvants using conventional formulation techniques. Suitable formulations and application methods are described in U.S. Patent No. 6,468,523.

[0087] "Pest" includes but is not limited to, insects, fungi, bacteria, nematodes, mites, ticks, and the like. Insect pests include insects selected from the orders *Coleoptera*, *Diptera*, *Hymenoptera*, *Lepidoptera*, *Mallophaga*, *Homoptera*, *Hemiptera*, *Orthoptera*, *Thysanoptera*, *Dermaptera*, *Isoptera*, *Anoplura*, *Siphonaptera*, *Trichoptera*, etc., particularly *Coleoptera*, *Lepidoptera*, and *Diptera*.

[0088] Insect pests of the invention for the major crops include: Maize: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Helicoverpa zea*, corn earworm; *Spodoptera frugiperda*, fall armyworm; *Diatraea grandiosella*, southwestern corn borer; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Diatraea saccharalis*, sugarcane borer; *Diabrotica virgifera*, western corn rootworm; *Diabrotica longicornis barberi*, northern corn rootworm; *Diabrotica undecimpunctata howardi*, southern corn rootworm; *Melanotus* spp., wireworms; *Cyclocephala borealis*, northern masked chafer (white grub); *Cyclocephala immaculata*, southern masked chafer (white grub); *Popillia japonica*, Japanese beetle; *Chaetocnema pulicaria*, corn flea beetle; *Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*, corn leaf aphid; *Anuraphis maidiradicis*, corn root aphid; *Blissus leucopterus leucopterus*, chinch bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Hylemya platura*, seedcorn maggot; *Agromyza parvicornis*, corn blot leafminer; *Anaphothrips obscurus*, grass thrips; *Solenopsis milesta*, thief ant; *Tetranychus urticae*, twospotted spider mite; Sorghum: *Chilo partellus*, sorghum borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Feltia subterranea*, granulate cutworm; *Phyllophaga crinita*, white grub; *Eleodes*, *Conoderus*, and *Aeolus* spp., wireworms; *Oulema melanopus*, cereal leaf beetle; *Chaetocnema pulicaria*, corn flea beetle; *Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*, corn leaf aphid; *Sipha flava*, yellow sugarcane aphid; *Blissus leucopterus leucopterus*, chinch bug; *Contarinia sorghicola*, sorghum midge; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, twospotted spider mite; Wheat: *Pseudaletia unipunctata*, army worm; *Spodoptera frugiperda*, fall armyworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Agrotis orthogollia*, western cutworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Oulema melanopus*, cereal leaf beetle; *Hypera punctata*, clover leaf weevil; *Diabrotica undecimpunctata howardi*, southern corn rootworm; Russian wheat aphid; *Schizaphis graminum*, greenbug; *Macrosiphum avenae*, English grain aphid; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Mayetiola destructor*, Hessian fly; *Sitodiplosis mosellana*, wheat midge; *Meromyza americana*, wheat stem maggot; *Hylemya coarctata*, wheat bulb fly; *Frankliniella fusca*, tobacco thrips; *Cephus cinctus*, wheat stem sawfly; *Aceria tulipae*, wheat curl mite; Sunflower: *Suleima helianthana*, sunflower bud moth; *Homoeosoma electellum*, sunflower moth; *zygogramma exclamationis*, sunflower beetle; *Bothyrus gibbosus*, carrot beetle; *Neolasioptera murtfeldtiana*, sunflower seed midge; Cotton: *Heliothis virescens*, cotton budworm; *Helicoverpa zea*, cotton bollworm; *Spodoptera exigua*, beet armyworm; *Pectinophora gossypiella*, pink bollworm; *Anthonomus grandis*, boll weevil; *Aphis gossypii*, cotton aphid; *Pseudatomoscelis seriatus*, cotton fleahopper; *Trialeurodes abutilonea*, bandedwinged whitefly; *Lygus lineolaris*, tarnished plant bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differenzialis*, differential grasshopper; *Thrips tabaci*, onion thrips; *Frankliniella fusca*, tobacco thrips; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, twospotted spider mite; Rice: *Diatraea saccharalis*, sugarcane borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Colaspis brunnea*, grape colaspis; *Lissorhoptrus oryzophilus*, rice water weevil; *Sitophilus oryzae*, rice weevil; *Nephotettix nigropictus*, rice leafhopper; *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug; Soybean: *Pseudoplusia includens*, soybean looper; *Anticarsia gemmatilis*, velvetbean caterpillar; *Platyphena scabra*, green cloverworm; *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Spodoptera exigua*, beet armyworm; *Heliothis virescens*, cotton budworm; *Helicoverpa zea*, cotton bollworm; *Epilachna varivestis*, Mexican bean beetle; *Myzus persicae*, green peach aphid; *Empoasca fabae*, potato leafhopper; *Acrosternum hilare*, green stink bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Hylemya platura*, seedcorn maggot; *Sericothrips variabilis*, soybean thrips; *Thrips tabaci*, onion thrips; *Tetranychus turkestanii*, strawberry spider mite; *Tetranychus urticae*, twospotted spider mite; Barley: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Schizaphis graminum*, greenbug; *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug; *Euschistus servus*, brown stink bug; *Delia platura*, seedcorn maggot; *Mayetiola destructor*, Hessian fly; *Petrobia latens*, brown wheat mite; Oil Seed Rape: *Brevicoryne brassicae*, cabbage aphid; *Phyllotreta cruciferae*, Flea beetle; *Mamestra configurata*, Bertha armyworm; *Plutella xylostella*, Diamond-back moth; *Delia* spp., Root maggots.

[0089] Nematodes include *Caenorhabditis elegans* and parasitic nematodes such as root-knot, cyst, and lesion nem-

atodes, including *Heterodera* spp., *Meloidogyne* spp., and *Globodera* spp.; particularly members of the cyst nematodes, including, but not limited to, *Heterodera glycines* (soybean cyst nematode); *Heterodera schachtii* (beet cyst nematode); *Heterodera avenae* (cereal cyst nematode); and *Globodera rostochiensis* and *Globodera pallida* (potato cyst nematodes). Lesion nematodes include *Pratylenchus* spp.

[0090] The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Example 1. Extraction of Plasmid DNA

[0091] A pure culture of strain ATX14875 was grown in large quantities of rich media. The culture was spun to harvest the cell pellet. The cell pellet was then prepared by treatment with SDS by methods known in the art, resulting in breakage of the cell wall and release of DNA. Proteins and large genomic DNA were then precipitated by a high salt concentration. The plasmid DNA was then precipitated by standard ethanol precipitation. The plasmid DNA was separated from any remaining chromosomal DNA by high-speed centrifugation through a cesium chloride gradient. The DNA was visualized in the gradient by UV light and the band of lower density (i.e. the lower band) was extracted using a syringe. This band contained the plasmid DNA from Strain ATX14875. The quality of the DNA was checked by visualization on an agarose gel.

Example 2. Cloning of Genes

[0092] The purified plasmid DNA was sheared into 5-10 kb sized fragments and the 5' and 3' single stranded overhangs repaired using T4 DNA polymerase and Klenow fragment in the presence of all four dNTPs. Phosphates were then attached to the 5' ends by treatment with T4 polynucleotide kinase. The repaired DNA fragments were then ligated overnight into a standard high copy vector (i.e. pBluescript SK+), suitably prepared to accept the inserts as known in the art (for example by digestion with a restriction enzyme producing blunt ends).

[0093] The quality of the library was analyzed by digesting a subset of clones with a restriction enzyme known to have a cleavage site flanking the cloning site. A high percentage of clones were determined to contain inserts, with an average insert size of 5-6 kb.

Example 3. High Throughput Sequencing of Library Plates

[0094] Once the shotgun library quality was checked and confirmed, colonies were grown in a rich broth in 2ml 96-well blocks overnight at 37°C at a shaking speed of 350 rpm. The blocks were spun to harvest the cells to the bottom of the block. The blocks were then prepared by standard alkaline lysis prep in a high throughput format.

[0095] The end sequences of clones from this library were then determined for a large number of clones from each block in the following way: The DNA sequence of each clone chosen for analysis was determined using the fluorescent dye terminator sequencing technique (Applied Biosystems, Foster City, CA) and standard primers flanking each side of the cloning site. Once the reactions had been carried out in the thermocycler, the DNA was precipitated using standard ethanol precipitation. The DNA was resuspended in water and loaded onto a capillary sequencing machine. Each library plate of DNA was sequenced from either end of the cloning site, yielding two reads per plate over each insert.

Example 4. Assembly and Screening of Sequencing Data

[0096] DNA sequences obtained were compiled into an assembly project and aligned together to form contigs. This can be done efficiently using a computer program, such as Vector NTi, or alternatively by using the Pred/Phrap suite of DNA alignment and analysis programs. These contigs, along with any individual read that may not have been added to a contig, were compared to a compiled database of all classes of known pesticidal genes. Contigs or individual reads identified as having identity to a known endotoxin or pesticidal gene were analyzed further. Among the sequences obtained, clones pAX018, pAX020, and pAX021 contained DNA identified as having homology to known endotoxin genes. Therefore, these clones were selected for further sequencing.

Example 5. Sequencing of pAX018, pAX020 and pAX021

[0097] Primers were designed to anneal to the clones of interest (pAX018, pAX020 and pAX021), in a manner such that DNA sequences generated from such primers will overlap existing DNA sequence of the clone(s). This process, known as "oligo walking", is well known in the art. This process was utilized to determine the entire DNA sequence of the region exhibiting homology to a known endotoxin gene. In the case of pAX021, this process was used to determine the DNA sequence of the entire clone, resulting in a single nucleotide sequence. The completed DNA sequence was

then placed back into the original large assembly for further validation. This allowed incorporation of more DNA sequence reads into the contig, resulting in multiple reads of coverage over the entire region.

[0098] Analysis of the DNA sequence of each clone by methods known in the art identified an open reading frame with homology to known delta endotoxin genes. The open reading frames found in pAX018, pAX020 and pAX021 were designated as AXMI-018, AXMI-020 and AXMI-021, respectively. The DNA sequence of AXMI-018 is provided as SEQ ID NO:1, and the amino acid sequence of the predicted protein is designated SEQ ID NO:2. The DNA sequence of AXMI-020 is provided as SEQ ID NO:3 and its predicted protein sequence is provided in SEQ ID NO: 4. The DNA sequence of AXMI-021 is provided as SEQ ID NO:5, and the amino acid sequence of the predicted protein is provided in SEQ ID NO:6.

Example 6. Homology Between AXMI-018, AXMI-020 and AXMI-021

[0099] The novel ORFs found in strain ATX14875 showed high homology to each other, with most changes observed near the toxic portion of the genes. AXMI-018 and AXMI-020 are full-length endotoxin genes, and contain a C-terminal non-toxic domain. AXMI-021 appears to be a naturally truncated endotoxin belonging to the same family. Figure 1 shows an alignment of the proteins, truncated to their predicted toxic portion. Table 1 shows the percent identity between the novel endotoxins at the amino acid level.

Table 1. Amino acid identity between AXMI-018, AXMI-020 and AXMI-021

	AXMI-018	AXMI-020	AXMI-021
AXMI-018	-	91%	97%
AXMI-020	91%	-	91%
AXMI-021	91%	91%	-

Example 7. Homology of Novel Genes to Known Endotoxin Genes

[0100] Searches of DNA and protein databases with the DNA sequence and amino acid sequence of AXMI-018, AXMI-020, and AXMI-021 reveal that they are homologous to a set of known endotoxins.

[0101] Figures 2A and 2B show an alignment of AXMI-018 with several endotoxins. Blast searches identify cry12Aa1 (Accession No. L07027) as having the strongest block of homology. However, alignment of the entire AMXI-018 protein (SEQ ID NO:2) to a large set of endotoxin proteins shows that AXMI-018 is most homologous to cry21Ba1 (Accession No. AB088406), and shares 25% amino acid identity with this toxin (see Table 2). The second column of Table 2 shows the amino acid identities of the untrimmed, full-length proteins. The third column of Table 2 reflects the homology of AXMI-018 within the toxin domains. The endotoxin with the highest homology through the N-terminal active portion of the gene is cry5Ab1 (Accession No. L07026). The amino acid identity of the truncated cry5Ab1 to the truncated AXMI-018 is 18% (see Table 2).

Table 2. Amino Acid Identity of AXMI-018 with Exemplary Endotoxin Classes

Endotoxin	Percent Amino Acid Identity to AXMI-018	Percent Amino Acid Identity of truncated Toxins to AXMI-018
<i>cry12Aa</i>	22.2%	16%
<i>cry21Aa</i>	23.7%	17%
<i>cry21Ba1</i>	25%	17%
<i>cry5Aa</i>	21.4%	17%
<i>cry5Ab</i>	23%	18%
<i>cry5Ba</i>	20.8%	17%
<i>cry1Ac</i>	17.5%	14%
<i>cry1Ba</i>	19%	14%
<i>cry1Ca</i>	18.6%	16%

[0102] Figures 3A and 3B show an alignment of AXMI-020 with several endotoxins. Blast searches identify cry12Aa1 (Accession No. L07027) as having the strongest block of homology. However, aligning the AMXI-020 protein (SEQ ID NO:4) to a large set of endotoxin proteins shows that the most homologous protein throughout the full length gene is actually cry21Ba1 (Accession No. AB088406), at 25% amino acid identity (see Table 3). The second column of Table 3 shows the amino acid identities of the untrimmed, full-length proteins. The third column reflects the true identity of the active portion of the protein by aligning only the toxic domains. The endotoxin with highest homology through the N-terminal active portion of the gene is cry5Ab1 (Accession No. L07026). The amino acid identity of the truncated cry5Ab1 to the truncated AXMI-020 is 18% (see Table 3).

Table 3. Amino Acid Identity of AXMI-020 with Exemplary Endotoxin Classes

Endotoxin	Percent Amino Acid Identity to AXMI-020	Percent Amino Acid Identity of truncated Toxins to AXMI-020
<i>cry12Aa</i>	24.1%	15%
<i>cry21Aa</i>	24.2%	17%
<i>cry21Ba1</i>	25%	17%
<i>cry5Aa</i>	21.9%	17%
<i>cry5Ab</i>	23.1%	18%
<i>cry5Ba</i>	22.6%	17%
<i>cry1Ac</i>	18.4%	14%
<i>cry1Ba</i>	19.7%	14%
<i>cry1Ca</i>	18.8%	16%

[0103] Figures 4A and 4B show an alignment of AXMI-021 with several endotoxins. Alignment of AMXI-021 protein (SEQ ID NO:6) to a large set of endotoxin proteins shows that the most homologous protein is cry5Ab1 (Accession No. L07026). The overall amino acid identity of the artificially truncated cry5Ab1 to AXMI-021 is 17% (see Table 4). Inspection of the amino acid sequence of AXMI-021 suggests that it does not contain a C-terminal non-toxic domain as is present in several endotoxin families. By removing this C-terminal protein of the toxins from the alignment, the alignment reflects the amino acid identity present solely in the toxin domains (see Table 4, column three). This "trimmed" alignment is shown in Figure 4A and 4B.

Table 4. Amino Acid Identity of AXMI-021 with Exemplary Endotoxin Classes

Endotoxin	Percent Amino Acid Identity to AXMI-021	Percent Amino Acid Identity of truncated Toxins to AXMI-021
<i>cry12Aa</i>	10.4%	15%
<i>cry21Aa</i>	11.6%	15%
<i>cry21Ba1</i>	10.2%	16%
<i>cry5Aa</i>	9.1%	16%
<i>cry5Ab</i>	11%	17%
<i>cry5Ba</i>	10.9%	14%
<i>cry1Ac</i>	9.9%	14%
<i>cry1Ba</i>	10.2%	14%
<i>cry1Ca</i>	9.8%	14%

[0104] Searches of the pFAM database identify AXMI-018, AXMI-020, and AXMI-021 as having homology to the delta endotoxin, N-terminal domain family (PFAM Accession No. PF03945). An Endotoxin_N domain is found between amino acid residues 70 and 302 of each protein (SEQ ID NOS:2, 4, and 6). An Endotoxin_C domain is found between amino acid residues 507 and 646 of each protein (SEQ ID NOS:2, 4, and 6).

[0105] This family contains insecticidal toxins produced by *Bacillus* species of bacteria. The N terminus of the crystalized

protein is cleaved after insect ingestion, resulting in an activated protein. The C terminal extension is cleaved in some protein members. This activated region of the delta endotoxin is composed of three structural domains. The N-terminal helical domain is involved in membrane insertion and pore formation. The second and third domains are involved in receptor binding.

Example 8. Expression of AXMI-018 and AXMI-021 in *Bacillus*

[0106] The insecticidal genes AXMI-018 and AXMI-021 are amplified by PCR from pAX018 and pAX021, respectively. The PCR products are cloned into the *Bacillus* expression vector pAX916 by methods well known in the art. The *Bacillus* strain containing the vector with either AXMI-018, designated pAX920, or AXMI-021, designated pAX931, is grown in CYS media (10 g/l Bacto-casitone; 3 g/l yeast extract; 6 g/l KH₂PO₄; 14 g/l K₂HPO₄; 0.5 mM MgSO₄; 0.05 mM MnCl₂; 0.05 mM FeSO₄), until sporulation is evident by microscopic examination. The resulting proteins are then tested for insecticidal activity in bioassays against important insect pests.

CYS Media

[0107] To prepare CYS media: 10 g/l Bacto-casitone; 3 g/l yeast extract; 6 g/l KH₂PO₄; 14 g/l K₂HPO₄; 0.5 mM MgSO₄; 0.05 mM MnCl₂; 0.05 mM FeSO₄. The CYS mix should be pH 7, if adjustment is necessary. NaOH or HCl are preferred. The media is then autoclaved and 100 ml of 10X filtered glucose is added after autoclaving. If the resultant solution is cloudy it can be stirred at room temperature to clear.

Example 9. Assay for Pesticidal Activity

[0108] The ability of a pesticidal protein to act as a pesticide upon a pest is often assessed in a number of ways. One way well known in the art is to perform a feeding assay. In such a feeding assay, one exposes the pest to a sample containing either compounds to be tested (i.e., compositions of the present invention), or control samples (samples not containing the test compound). Often this is performed by placing the material to be tested, or a suitable dilution of such material, onto a material that the pest will ingest, such as an artificial diet. The material to be tested may be composed of a liquid, solid, or slurry. The material to be tested may be placed upon the surface and then allowed to dry. Alternatively, the material to be tested may be mixed with a molten artificial diet, then dispensed into the assay chamber. The assay chamber may be, for example, a cup, a dish, or a well of a microtiter plate.

[0109] Assays for sucking pests (for example aphids) may involve separating the test material from the insect by a partition, ideally a portion that can be pierced by the sucking mouth parts of the sucking insect, to allow ingestion of the test material. Often the test material is mixed with a feeding stimulant, such as sucrose, to promote ingestion of the test compound.

[0110] Other types of assays can include microinjection of the test material into the mouth, or gut of the pest, as well as development of transgenic plants, followed by test of the ability of the pest to feed upon the transgenic plant. Plant testing may involve isolation of the plant parts normally consumed, for example, small cages attached to a leaf, or isolation of entire plants in cages containing insects.

[0111] Other methods and approaches to assay pests are known in the art, and can be found, for example in Robertson, J. L. & H. K. Preisler (1992), Pesticide bioassays with arthropods, CRC, Boca Raton, FL. Alternatively, assays are commonly described in the journals *Arthropod Management Tests* and *Journal of Economic Entomology* or by discussion with members of the Entomological Society of America (ESA).

Example 10. *C. elegans* bioassay

[0112] The activity of a pesticidal protein(s) upon the nematode *Caenorhabditis elegans* (*C. elegans*) is a useful predictor of general nematocidal activity. *C. elegans* hermaphrodites are reared as known in the art, to generate populations of healthy animals for bioassay. General procedures for growth, harvesting, and genetic manipulation of *C. elegans* including growth media, etc., may be found in the art, for example, in Wood, ed. (1988) *The Nematode Caenorhabditis elegans*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

[0113] Sterile supernatants from organisms such as those expressing the polypeptides of the invention may be tested for activity upon *C. elegans*. Bioassays are performed in 96-well plates. For the test samples, five to ten nematodes are added to, for example, 80 µl of S medium (Woods, 1998, *supra*) and mixed with, for example, 20 µl of sterile supernatant, and 0.5 µl of concentrated HB101 (prepared as described in Woods, 1998, *supra*) and rifampicin (final concentration of 0.1 µg/µl). Assays are allowed to proceed at room temperature for 3 days, and the effects of the test compound on the *C. elegans* organisms are recorded.

Example 11. Vectoring of AXMI-018, AXMI-020 and AXMI-021 for Plant Expression

[0114] The coding regions of AXMI-018, AXMI-020, and AXMI-021 are operably connected with appropriate promoter and terminator sequences for expression in plants. Such sequences are well known in the art and may include the rice actin promoter or maize ubiquitin promoter for expression in monocots, the *Arabidopsis* UBQ3 promoter or CaMV 35S promoter for expression in dicots, and the nos or PinII terminators. Techniques for producing and confirming promoter - gene - terminator constructs also are well known in the art.

[0115] The plant expression cassettes described above are combined with an appropriate plant selectable marker to aid in the selection of transformed cells and tissues, and ligated into plant transformation vectors. These vectors may include binary vectors from *Agrobacterium*-mediated transformation or simple plasmid vectors for aerosol or biolistic transformation.

Example 12. Transformation of Maize Cells with AXMI-018, AXMI-020 and AXMI-021

[0116] Maize ears are best collected 8-12 days after pollination. Embryos are isolated from the ears, and those embryos 0.8-1.5 mm in size are preferred for use in transformation. Embryos are plated scutellum side-up on a suitable incubation media, such as DN62A5S media (3.98 g/L N6 Salts; 1 mL/L (of 1000x Stock) N6 Vitamins; 800 mg/L L-Asparagine; 100 mg/L Myo-inositol; 1.4 g/L L-Proline; 100 mg/L Casamino acids; 50 g/L sucrose; 1 mL/L (of 1 mg/mL Stock) 2,4-D). However, media and salts other than DN62A5S are suitable and are known in the art. Embryos are incubated overnight at 25°C in the dark. However, it is not necessary *per se* to incubate the embryos overnight.

[0117] The resulting explants are transferred to mesh squares (30-40 per plate), transferred onto osmotic media for about 30-45 minutes, then transferred to a beaming plate (see, for example, PCT Publication No. WO/0138514 and U.S. Patent No. 5,240,842).

[0118] DNA constructs designed to express the pesticidal polypeptides of the invention in plant cells are accelerated into plant tissue using an aerosol beam accelerator, using conditions essentially as described in PCT Publication No. WO/0138514. After beaming, embryos are incubated for about 30 min on osmotic media, then placed onto incubation media overnight at 25°C in the dark. To avoid unduly damaging beamed explants, they are incubated for at least 24 hours prior to transfer to recovery media. Embryos are then spread onto recovery period media, for about 5 days at 25°C in the dark, then transferred to selection media. Explants are incubated in selection media for up to eight weeks, depending on the nature and characteristics of the particular selection utilized. After the selection period, the resulting callus is transferred to embryo maturation media, until the formation of mature somatic embryos is observed. The resulting mature somatic embryos are then placed under low light, and the process of regeneration is initiated by methods known in the art. The resulting shoots are allowed to root on rooting media, and the resulting plants are transferred to nursery pots and propagated as transgenic plants.

*Materials***DN62A5S Media**

[0119]

Components	per liter	Source
Chu's N6 Basal Salt Mixture (Prod. No. C 416)	3.98 g/L	Phytotechnology Labs
Chu's N6 Vitamin Solution (Prod. No. C 149)	1 mL/L (of 1000x Stock)	Phytotechnology Labs
L-Asparagine	800 mg/L	Phytotechnology Labs
Myo-inositol	100 mg/L	Sigma
L-Proline	1.4 g/L	Phytotechnology Labs
Casamino acids	100 mg/L	Fisher Scientific
Sucrose	50 g/L	Phytotechnology Labs
2,4-D (Prod. No. D-7299)	1 mL/L (of 1 mg/mL Stock)	Sigma

[0120] The pH of the solution is adjusted to pH 5.8 with 1N KOH/1N KCl, Gelrite (Sigma) up to 3g/L is added, and the mixture is autoclaved. After cooling to 50°C, 2 ml/L of a 5 mg/ml stock solution of Silver Nitrate (Phytotechnology Labs)

is added. The recipe yields about 20 plates.

Example 13. Transformation of AXMI-018, AXMI-020 and AXMI-021 into Plant Cells by *Agrobacterium*-Mediated Transformation

[0121] Ears are best collected 8-12 days after pollination. Embryos are isolated from the ears, and those embryos 0.8-1.5 mm in size are preferred for use in transformation. Embryos are plated scutellum side-up on a suitable incubation medium, and incubated overnight at 25°C in the dark. However, it is not necessary *per se* to incubate the embryos overnight. Embryos are contacted with an *Agrobacterium* strain containing the appropriate vectors for Ti plasmid mediated transfer for about 5-10 min, and then plated onto co-cultivation media for about 3 days (25°C in the dark). After co-cultivation, explants are transferred to recovery period media for about five days (at 25°C in the dark). Explants are incubated in selection media for up to eight weeks, depending on the nature and characteristics of the particular selection utilized. After the selection period, the resulting callus is transferred to embryo maturation media, until the formation of mature somatic embryos is observed. The resulting mature somatic embryos are then placed under low light, and the process of regeneration is initiated as known in the art. The resulting shoots are allowed to root on rooting media, and the resulting plants are transferred to nursery pots and propagated as transgenic plants.

[0122] All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains.

SEQUENCE LISTING

[0123]

<110> Nadine Carozzi

Tracy Hargiss

Michael G. Koziel

Nicholas B. Duck

<120> AXMI-018, AXMI-020, and AXMI-021, A

Family of Delta-Endotoxin Genes and Methods for Their Use

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	Ile	Met	Leu	Thr	Ala	Asn	Ala	Ser	Gln	Asn	Val	Phe	Ile	Asp	Arg	Ile	625	630	635	640
	Glu	Phe	Val	Pro	Ile	Ala	Thr	Thr	Glu	Pro	Val	Thr	Ile	Pro	Asn	Thr				
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	Thr	Ala	Gln	Pro	Gly	Ile	Leu	Gly	Asp	Ile	Val	Asn	Leu	Ser	Gly	Tyr				
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	Ile	Gln	Phe	Phe	Arg	Asn	Asn	Gln	Leu	Val	Asp	His	Tyr	Asp	Thr	Ser				
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	Glu	Gly	Arg	Tyr	Pro	His	Asn	Ala	Asp	Phe	Asn	Met	Ser	Asn	Tyr	Lys				
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	Tyr	Thr	Glu	Pro	Val	Glu	Gly	Gln	Ile	Ser	Gly	Thr	Ile	Thr	Leu	Ile				
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	Glu	Ser	Lys	Leu	Lys	Pro	Tyr	Thr	Arg	Tyr	Ile	Val	Arg	Gly	Phe	Ile				
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	Gly	Ala	Leu	Gln	Leu	Glu	Ser	Asn	Leu	Gly	Ile	Glu	Ile	Gly	Phe	Lys				
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	Glu	Lys	Gln	Trp	Leu	Arg	Leu	Ser	Gln	Lys	Gln	Gln	Ser	Gln	Leu	Gln				
	1025				1030						1035				1040					
50	Lys	Gln	Tyr	Asp	Gln	Thr	Met	Gln	Tyr	Phe	Ala	Thr	Leu	Tyr	Thr	Thr				
				1045						1050					1055					
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55	Gln	Leu	Ser	Arg	Thr	Ser	Ser	Pro	Met	Ile	Glu	Glu	Leu	Val	Arg	Thr				
				1090			1095						1100							

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Lys Glu Lys Ala Leu Gln Leu Tyr Pro Thr Asn Val Ile Gln Asn Gly
 1105 1110 1115 1120
 Asn Phe Ser Ser Gly Leu Ser Thr Trp His Val Ile Glu Asn Thr Asn
 1125 1130 1135
 Val Arg Ile Glu Phe Ile Asn Gly Ile Ser Val Leu His Val Pro Ser
 1140 1145 1150
 Trp Asp Glu Thr Val Ser Gln Thr Ile Thr Leu Pro Pro His Gln Glu
 1155 1160 1165
 Asn Ile Leu Tyr Gln Leu Arg Val Thr Ala Lys Gly Asn Gly Ser Val
 1170 1175 1180
 Ile Leu Gln His Asn Gly Glu Gln Glu Arg Leu Tyr Phe Asp Gln Asn
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 gta tct act tta gaa tca tct tta att cct tac aat gtg tac gcg aca 96
 Val Ser Thr Leu Glu Ser Ser Leu Ile Pro Tyr Asn Val Tyr Ala Thr
 20 25 30
 gat cag ttt aac tct aat aaa aat tgg gaa gat gca ctg aaa aaa tta 144
 Asp Gln Phe Asn Ser Asn Lys Asn Trp Glu Asp Ala Leu Lys Lys Leu
 35 40 45
 tta gaa aaa ttt tat tcc ggt gat tta aca cag gat gct att gat att 192
 Leu Glu Lys Phe Tyr Ser Gly Asp Leu Thr Gln Asp Ala Ile Asp Ile
 50 55 60
 ttt ctt ggt gac agc ggc ttt gat tac tta tct tta gta aat gtt att 240
 Phe Leu Gly Asp Ser Gly Phe Asp Tyr Leu Ser Leu Val Asn Val Ile
 65 70 75 80
 ttt tct att gca gga tct ttt att cct tat gtg ggt gct ctt gtc cct 288
 Phe Ser Ile Ala Gly Ser Phe Ile Pro Tyr Val Gly Ala Leu Val Pro
 85 90 95
 atc att aat ctt ctt ttt gga tca gag agc aaa cca gat gta ttt gaa 336
 Ile Ile Asn Leu Leu Phe Gly Ser Glu Ser Lys Pro Asp Val Phe Glu
 100 105 110
 caa atg aga gca cga att gaa gca tta att cat aag gaa tta tct gca 384
 Gln Met Arg Ala Arg Ile Glu Ala Leu Ile His Lys Glu Leu Ser Ala
 115 120 125

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	gac cat gtg caa aca tta aaa gca gaa att aag gga ctt aaa gat acg	432
	Asp His Val Gln Thr Leu Lys Ala Glu Ile Lys Gly Leu Lys Asp Thr	
	130 135 140	
5	gga gat cta tat caa aaa gat gta aat gct gtt gca gga aga aca aat	480
	Gly Asp Leu Tyr Gln Lys Asp Val Asn Ala Val Ala Gly Arg Thr Asn	
	145 150 155 160	
10	gga cct acc cct cca tca ttt gat agc aat aca gat gct tta aaa gca	528
	Gly Pro Thr Pro Pro Ser Phe Asp Ser Asn Thr Asp Ala Leu Lys Ala	
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	Glu Leu Arg Ser Gln Ile Thr Ala Thr Asn Thr Leu Phe Val Gln Arg	
	180 185 190	
20	atg cct caa ttt gct ata gag gga tat gaa gag att act cta cct tta	624
	Met Pro Gln Phe Ala Ile Glu Gly Tyr Glu Glu Ile Thr Leu Pro Leu	
	195 200 205	
25	cac act atc gct gca agt atg cat ctt ata ttc tta aaa gat gtt tgt	672
	His Thr Ile Ala Ala Ser Met His Leu Ile Phe Leu Lys Asp Val Cys	
	210 215 220	
30	gaa cat ggt gct gaa tgg gga att gct aat act aca tta aca aat tat	720
	Glu His Gly Ala Glu Trp Gly Ile Ala Asn Thr Thr Leu Thr Asn Tyr	
	225 230 235 240	
35	caa ggt caa tta caa gat tgt att aga gag tat tca aat aaa gct tat	768
	Gln Gly Gln Leu Gln Asp Cys Ile Arg Glu Tyr Ser Asn Lys Ala Tyr	
	245 250 255	
40	tcg atg ttc aat att ggt tta cag agg gca aaa aat aat gga aac aat	816
	Ser Met Phe Asn Ile Gly Leu Gln Arg Ala Lys Asn Asn Gly Asn Asn	
	260 265 270	
45	atg tgg aat aac gta aat aac tat atc cgc aca atg aaa tta aat gct	864
	Met Trp Asn Asn Val Asn Asn Tyr Ile Arg Thr Met Lys Leu Asn Ala	
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	Leu Asp Thr Val Ala Gln Trp Pro Ile Leu Asp Lys Val Thr Tyr Pro	
	290 295 300	
55	tta gat aca aca tta caa caa aca cgc ggt ata ttt tca gat cta tca	960
	Leu Asp Thr Thr Leu Gln Gln Thr Arg Gly Ile Phe Ser Asp Leu Ser	
	305 310 315 320	
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	Gly Arg Gly Gly Thr Gln Ser Asn Tyr Arg Tyr Asp Tyr Asp Ala Val	
	325 330 335	
65	caa ggt tat gct cct tct ttt gtc gga ttt gat acc gaa cta aat gtt	
	Gln Gly Tyr Ala Pro Ser Phe Val Gly Phe Asp Thr Glu Leu Asn Val	
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Val Asn Asp Phe Gly Tyr Lys Asp Leu Thr Ala Ile Gln Thr Phe Thr
355 360 365

5 ggt gat cga att gat tca att tgg caa tca ttt aag tat aat tca gga
1152
Gly Asp Arg Ile Asp Ser Ile Trp Gln Ser Phe Lys Tyr Asn Ser Gly
370 375 380

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1200
Glu Pro Phe Leu Thr Asn Leu Gly Asn Gly Lys Arg Gly Asn Asn Pro
385 390 395 400

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1248
Val Ile Pro Asn Ser Arg Asp Asn Pro Ile Ile Ser Ala Lys Gly Ser
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20 aga cca tct gca aac tat gtt ggg atg aat ttc caa cga gca gat aaa
1296
Arg Pro Ser Ala Asn Tyr Val Gly Met Asn Phe Gln Arg Ala Asp Lys
420 425 430

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1344
Thr Val Val Pro Asn Gly Tyr Val Ile Pro Asn Asp Asn Tyr Thr Val
435 440 445

30 ccc gct ggg cat aaa ctt gga tgg att tca gcc ctg cat gat gaa tta
1392
Pro Ala Gly His Lys Leu Gly Trp Ile Ser Ala Leu His Asp Glu Leu
450 455 460

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1440
Asp Asn Ala Asn Asn Ala Asp Leu Val Val Ser Val Trp Val Lys Asn
465 470 475 480

40 gat atc ttc cag gaa aat att atc ggt tcc ata aaa aca gtt act act
1488
Asp Ile Phe Gln Glu Asn Ile Ile Gly Ser Ile Lys Thr Val Thr Thr
485 490 495

45 gat gat gga acc aca gaa aat aga caa caa att ata ggg atc ccg gca
1536
Asp Asp Gly Thr Thr Glu Asn Arg Gln Gln Ile Ile Gly Ile Pro Ala
500 505 510

50 gat aaa cat atg aca aga agt aca aag cga atg gaa ctg gaa ttt atc
1584
Asp Lys His Met Thr Arg Ser Thr Lys Arg Met Glu Leu Glu Phe Ile
515 520 525

55 aat ggt aca aat ggg tca atg agc tta tct agt act aat gat caa ttg
1632
Asn Gly Thr Asn Gly Ser Met Ser Leu Ser Ser Thr Asn Asp Gln Leu
530 535 540

tat tat acg att aat cct ata gtt agc cag aga tat caa att cgg tat
1680

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Tyr Tyr Thr Ile Asn Pro Ile Val Ser Gln Arg Tyr Gln Ile Arg Tyr
 545 550 555 560
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 1728
 Arg Val Ala Thr Thr Ser Ala Glu Ser Leu Asp Leu Trp Ile Asp Gly
 565 570 575
 10 tat aaa cgc gga aca acc ccg tta cca aat aca agt agc aca tca acg
 1776
 Tyr Lys Arg Gly Thr Thr Pro Leu Pro Asn Thr Ser Ser Thr Ser Thr
 580 585 590
 15 caa aca caa aaa gtg ata att caa ggg tta caa gga aaa tat caa tta
 1824
 Gln Thr Gln Lys Val Ile Ile Gln Gly Leu Gln Gly Lys Tyr Gln Leu
 595 600 605
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 1872
 Ile Asn Gly Pro Ile Leu Asp Leu Thr Ala Gly Ser His Thr Phe Gly
 610 615 620
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 Ile Ala Leu Thr Ala Thr Pro Ser Gln Asn Val Phe Ile Asp Arg Ile
 625 630 635 640
 30 gaa ttt gtt cct ata ggg tca cct tgc cag aat ata ttt cct gct ggt
 1968
 Glu Phe Val Pro Ile Gly Ser Pro Cys Gln Asn Ile Phe Pro Ala Gly
 645 650 655
 35 cca ttt aca gta gat aat gga aga aaa aca gtt tgg act tcc tcg aca
 2016
 Pro Phe Thr Val Asp Asn Gly Arg Lys Thr Val Trp Thr Ser Ser Thr
 660 665 670
 40 gga aca gcc ttt tca gta gaa aat att caa gga ttt gtg gga atg aga
 2064
 Gly Thr Ala Phe Ser Val Glu Asn Ile Gln Gly Phe Val Gly Met Arg
 675 680 685
 45 aat ttt aat tgg cgt att gaa ttt tta caa aaa ggg gtt act tta tct
 2112
 Asn Phe Asn Trp Arg Ile Glu Phe Leu Gln Lys Gly Val Thr Leu Ser
 690 695 700
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 2160
 Gln Tyr Thr Ile Pro Ile Thr Gly Ala Ser Phe Asp His Tyr Ser Phe
 705 710 715 720
 55 ggc cct ttt tct aaa gac ata cct gaa gga ttt gat acg att caa atc
 2208
 Gly Pro Phe Ser Lys Asp Ile Pro Glu Gly Phe Asp Thr Ile Gln Ile
 725 730 735
 gta tct ccc gat ttt ccg ata gtt ata acg cct att gat gga aaa gtc
 2256

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	2304															
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	2352															
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	2400															
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	785					790					795					800
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	2448															
	Val	Asn	Ala	Leu	Ser	Asp	Asp	Leu	Phe	Gly	Thr	Glu	Lys	Glu	Arg	Leu
					805					810					815	
25	cgc	caa	cgt	atg	gct	cgg	gct	aag	caa	cta	aat	aat	aca	aaa	aat	ata
	2496															
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	2592															
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	2640															
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	865					870					875					880
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	2688															
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	2736															
	Arg	Gly	Phe	Ile	Gly	Glu	Ala	Glu	Asp	Leu	Ala	Leu	Met	Val	Ser	Arg
				900					905					910		
55	tat	ggg	aaa	gaa	att	gat	aca	gct	ttt	acg	ggt	cct	tat	caa	gaa	gcg
	2784															
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			915					920					925			
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	2832															

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	Leu	Pro	Leu	Ser	Pro	Asp	Ser	Ser	Ser	Asn	Cys	Cys	Gly	Pro	Val	Ala	
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	2928																
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	3072																
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		1010					1015					1020					
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	3120																
	Ser	Gln	Leu	Gln	Lys	Gln	Tyr	Asp	Gln	Thr	Met	Gln	Tyr	Phe	Ala	Thr	
	1025				1030						1035					1040	
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	Thr	Asp	Ile	Ala	Asn	Val	Gln	Val	Ile	Thr	Phe	Pro	Ser	Thr	Met	Gln	
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		1090				1095						1100					
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55	gaa	aat	aca	aac	gta	cgt	ata	gag	ttc	att	aat	ggg	ata	tct	gta	tta	
	3408																

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Glu Asn Thr Asn Val Arg Ile Glu Phe Ile Asn Gly Ile Ser Val Leu
 1125 1130 1135
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 3456
 His Val Pro Ser Trp Asp Glu Thr Val Ser Gln Thr Ile Thr Leu Pro
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 3504
 Pro His Gln Glu Asn Ile Leu Tyr Gln Leu Arg Val Thr Ala Lys Gly
 1155 1160 1165
 aat ggt agt gtt atc ctt cag cat aat ggc gaa caa gaa aga cta tat
 3552
 Asn Gly Ser Val Ile Leu Gln His Asn Gly Glu Gln Glu Arg Leu Tyr
 1170 1175 1180
 ttc gat caa aat aat tca aca gga aat act ttt gta aca aaa gaa att
 3600
 Phe Asp Gln Asn Asn Ser Thr Gly Asn Thr Phe Val Thr Lys Glu Ile
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 3648
 Ser Phe Tyr Pro Thr Ala Ser Thr Leu Ser Leu Gln Ile Gln Ser Glu
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 Pro Leu Thr *
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<400> 4

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5	Asp	Gln	Phe	Asn	Ser	Asn	Lys	Asn	Trp	Glu	Asp	Ala	Leu	Lys	Lys	Leu
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	Leu	Glu	Lys	Phe	Tyr	Ser	Gly	Asp	Leu	Thr	Gln	Asp	Ala	Ile	Asp	Ile
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	Phe	Leu	Gly	Asp	Ser	Gly	Phe	Asp	Tyr	Leu	Ser	Leu	Val	Asn	Val	Ile
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			100					105					110			
15	Gln	Met	Arg	Ala	Arg	Ile	Glu	Ala	Leu	Ile	His	Lys	Glu	Leu	Ser	Ala

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		115				120				125						
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	Gln	Gly	Tyr	Ala	Pro	Ser	Phe	Val	Gly	Phe	Asp	Thr	Glu	Leu	Asn	Val
			340						345					350		
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35	Arg	Pro	Ser	Ala	Asn	Tyr	Val	Gly	Met	Asn	Phe	Gln	Arg	Ala	Asp	Lys
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	Thr	Val	Val	Pro	Asn	Gly	Tyr	Val	Ile	Pro	Asn	Asp	Asn	Tyr	Thr	Val
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45	Asp	Asp	Gly	Thr	Glu	Asn	Arg	Gln	Gln	Ile	Ile	Gly	Ile	Pro	Ala	
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	Asp	Lys	His	Met	Thr	Arg	Ser	Thr	Lys	Arg	Met	Glu	Leu	Glu	Phe	Ile
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	Ala	Lys	Val	Thr	Ala	Val	Val	Asn	Ala	Leu	Phe	Ile	Thr	Asp	Thr	Gln
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	785				790						795				800	
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	Leu	Glu	Ile	Val	Glu	Asp	Arg	Ser	Leu	Thr	Glu	Ala	Glu	Thr	Ile	Lys
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	1025					1030					1035				1040	
	Leu	Tyr	Thr	Thr	Ser	Asp	Gln	Thr	Glu	Leu	Lys	Asn	Thr	Val	Gln	Tyr
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55	Trp	Phe	Ile	Pro	Gln	Leu	Arg	Arg	Thr	Ser	Ser	Pro	Met	Ile	Glu	Glu

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 1090 1095 1100
 5 Ile Gln Asn Gly Asn Phe Ser Ser Gly Leu Ser Thr Trp His Val Ile
 1105 1110 1115 1120
 Glu Asn Thr Asn Val Arg Ile Glu Phe Ile Asn Gly Ile Ser Val Leu
 1125 1130 1135
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 Val Ser Thr Leu Glu Ser Ser Leu Ile Pro Tyr Asn Val Tyr Ala Thr
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 Asp Gln Phe Asn Ser Asn Lys Asn Trp Glu Asp Ala Leu Lys Lys Leu
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 Phe Ser Ile Ala Gly Ser Phe Ile Pro Tyr Val Gly Ala Leu Val Pro
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 55 atc att aat ctt ctt ttt gga tca gag agc aaa cca gat gta ttt gaa 336
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	115 120 125	
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	Asp His Val Gln Thr Leu Lys Ala Glu Ile Lys Gly Leu Lys Asp Thr	
	130 135 140	
10	gga gat cta tat caa aaa gat gta aat gct gtt gca gga aga aca aat	480
	Gly Asp Leu Tyr Gln Lys Asp Val Asn Ala Val Ala Gly Arg Thr Asn	
	145 150 155 160	
15	gga cct acc cct cca tca ttt gat agc aat aca gat gct tta aaa gca	528
	Gly Pro Thr Pro Pro Ser Phe Asp Ser Asn Thr Asp Ala Leu Lys Ala	
	165 170 175	
	gaa ctt cga agt caa atc aca gct aca aac act cta ttt gtg caa cga	576
	Glu Leu Arg Ser Gln Ile Thr Ala Thr Asn Thr Leu Phe Val Gln Arg	
	180 185 190	
20	atg cct caa ttt gct ata gag gga tat gaa gag att act cta cct tta	624
	Met Pro Gln Phe Ala Ile Glu Gly Tyr Glu Glu Ile Thr Leu Pro Leu	
	195 200 205	
25	cac act atc gct gca agt atg cat ctt ata ttc tta aaa gat gtt tgt	672
	His Thr Ile Ala Ala Ser Met His Leu Ile Phe Leu Lys Asp Val Cys	
	210 215 220	
30	gaa cat ggt gct gaa tgg gga att gct aat act aca tta aca aat tat	720
	Glu His Gly Ala Glu Trp Gly Ile Ala Asn Thr Thr Leu Thr Asn Tyr	
	225 230 235 240	
	caa ggt caa tta caa gat tgt att aga gag tat tca aat aaa gct tat	768
	Gln Gly Gln Leu Gln Asp Cys Ile Arg Glu Tyr Ser Asn Lys Ala Tyr	
	245 250 255	
35	tcg atg ttc aat att ggt tta cag agg gca aaa aat aat gga aac aat	816
	Ser Met Phe Asn Ile Gly Leu Gln Arg Ala Lys Asn Asn Gly Asn Asn	
	260 265 270	
40	atg tgg aat aac gta aat aac tat atc cgc aca atg aaa tta aat gct	864
	Met Trp Asn Asn Val Asn Asn Tyr Ile Arg Thr Met Lys Leu Asn Ala	
	275 280 285	
45	tta gat act gtt gct caa tgg cct att ctg gat aaa gta aca tac cca	912
	Leu Asp Thr Val Ala Gln Trp Pro Ile Leu Asp Lys Val Thr Tyr Pro	
	290 295 300	
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	Leu Asp Thr Thr Leu Gln Gln Thr Arg Gly Ile Phe Ser Asp Leu Ser	
	305 310 315 320	
55	ggt agg ggg ggg aca caa tct aat tat aga tat gat tat gat gct gtt	1008
	Gly Arg Gly Gly Thr Gln Ser Asn Tyr Arg Tyr Asp Tyr Asp Ala Val	
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340 345 350

5 gta aac gat ttt ggt tat aaa gat tta acc gca att cag aca ttt aca
1104
Val Asn Asp Phe Gly Tyr Lys Asp Leu Thr Ala Ile Gln Thr Phe Thr
355 360 365

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1152
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370 375 380

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1200
Glu Pro Phe Leu Thr Asn Leu Gly Asn Gly Lys Pro Gly Asn Asn Pro
385 390 395 400

20 gtg att cca aat agc aga gat aat ccg att att tcc gca aaa gga tct
1248
Val Ile Pro Asn Ser Arg Asp Asn Pro Ile Ile Ser Ala Lys Gly Ser
405 410 415

25 aga cca tct gca aac tat gtt ggg atg aat ttc caa cga gca aat aaa
1296
Arg Pro Ser Ala Asn Tyr Val Gly Met Asn Phe Gln Arg Ala Asn Lys
420 425 430

30 act gta gtt tca aat gga tat gta att cct aat gac aat tat aca gta
1344
Thr Val Val Ser Asn Gly Tyr Val Ile Pro Asn Asp Asn Tyr Thr Val
435 440 445

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1392
Pro Ala Gly His Lys Leu Gly Trp Ile Ser Ala Leu His Asp Glu Leu
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1440
Asp Asn Ala Asn Asn Ala Asp Leu Val Val Ser Val Trp Val Lys Asn
465 470 475 480

45 gat atc ttc cag gaa aat att atc ggt tcc ata aaa aca gtt act act
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1536
Asp Asp Gly Thr Thr Glu Asn Arg Gln Gln Ile Ile Gly Ile Pro Ala
500 505 510

55 gat aaa cat atg aca aga agt aca aag cga atg gaa ctg gaa ttt atc
1584
Asp Lys His Met Thr Arg Ser Thr Lys Arg Met Glu Leu Glu Phe Ile
515 520 525

aat ggt aca aat ggg tca atg agc tta tct agt act aat gat caa ttg
1632

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	Tyr Tyr Thr Ile Asn Pro Ile Val Ser Gln Arg Tyr Gln Ile Arg Tyr	
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10	cgc gta gca aca act tca gca gaa tct tta gac cta tgg atc gat ggt	
	1728	
	Arg Val Ala Thr Thr Ser Ala Glu Ser Leu Asp Leu Trp Ile Asp Gly	
	565 570 575	
15	tat aaa cgc gga aca acc ccg tta cca aat aca agt agc aca tca acg	
	1776	
	Tyr Lys Arg Gly Thr Thr Pro Leu Pro Asn Thr Ser Ser Thr Ser Thr	
	580 585 590	
20	caa aca caa aaa gtg ata att caa ggg tta caa gga aaa tat caa tta	
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	Gln Thr Gln Lys Val Ile Ile Gln Gly Leu Gln Gly Lys Tyr Gln Leu	
	595 600 605	
25	att aat gga cca act ctt gat ttg aca gca ggt tcc cat act ttt ggt	
	1872	
	Ile Asn Gly Pro Thr Leu Asp Leu Thr Ala Gly Ser His Thr Phe Gly	
	610 615 620	
30	att atg tta aca gca aat gct tct caa aat gta ttt att gat cgc att	
	1920	
	Ile Met Leu Thr Ala Asn Ala Ser Gln Asn Val Phe Ile Asp Arg Ile	
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35	gaa ttt gtt cct ata gct aca aca gaa cct gtc aca ata ccc aat aca	
	1968	
	Glu Phe Val Pro Ile Ala Thr Thr Glu Pro Val Thr Ile Pro Asn Thr	
	645 650 655	
40	cct att aaa act tat aca aat cca cca aat cct caa caa gta ctt tgg	
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	Pro Ile Lys Thr Tyr Thr Asn Pro Pro Asn Pro Gln Gln Val Leu Trp	
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45	act gct cag cca ggt att ttg ggt gat ata gta aat tat cat atc aac	
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	Thr Ala Gln Pro Gly Ile Leu Gly Asp Ile Val Asn Tyr His Ile Asn	
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	Val	Ser	Thr	Leu	Glu	Ser	Ser	Leu	Ile	Pro	Tyr	Asn	Val	Tyr	Ala	Thr
5				20					25					30		
	Asp	Gln	Phe	Asn	Ser	Asn	Lys	Asn	Trp	Glu	Asp	Ala	Leu	Lys	Lys	Leu
			35					40					45			
	Leu	Glu	Lys	Phe	Tyr	Ser	Gly	Asp	Leu	Thr	Gln	Asp	Ala	Ile	Asp	Ile
		50					55					60				
	Phe	Leu	Gly	Asp	Ser	Gly	Phe	Asp	Tyr	Leu	Ser	Leu	Val	Asn	Val	Ile
10	65					70					75					80
	Phe	Ser	Ile	Ala	Gly	Ser	Phe	Ile	Pro	Tyr	Val	Gly	Ala	Leu	Val	Pro
					85					90					95	
	Ile	Ile	Asn	Leu	Leu	Phe	Gly	Ser	Glu	Ser	Lys	Pro	Asp	Val	Phe	Glu
				100					105					110		
15	Gln	Met	Arg	Ala	Arg	Ile	Glu	Ala	Leu	Ile	His	Lys	Glu	Leu	Ser	Ala
			115					120					125			
	Asp	His	Val	Gln	Thr	Leu	Lys	Ala	Glu	Ile	Lys	Gly	Leu	Lys	Asp	Thr
		130					135					140				
	Gly	Asp	Leu	Tyr	Gln	Lys	Asp	Val	Asn	Ala	Val	Ala	Gly	Arg	Thr	Asn
	145					150					155					160
20	Gly	Pro	Thr	Pro	Pro	Ser	Phe	Asp	Ser	Asn	Thr	Asp	Ala	Leu	Lys	Ala
					165					170					175	
	Glu	Leu	Arg	Ser	Gln	Ile	Thr	Ala	Thr	Asn	Thr	Leu	Phe	Val	Gln	Arg
				180					185					190		
	Met	Pro	Gln	Phe	Ala	Ile	Glu	Gly	Tyr	Glu	Glu	Ile	Thr	Leu	Pro	Leu
			195					200					205			
25	His	Thr	Ile	Ala	Ala	Ser	Met	His	Leu	Ile	Phe	Leu	Lys	Asp	Val	Cys
		210					215					220				
	Glu	His	Gly	Ala	Glu	Trp	Gly	Ile	Ala	Asn	Thr	Thr	Leu	Thr	Asn	Tyr
	225					230					235					240
	Gln	Gly	Gln	Leu	Gln	Asp	Cys	Ile	Arg	Glu	Tyr	Ser	Asn	Lys	Ala	Tyr
30					245					250					255	
	Ser	Met	Phe	Asn	Ile	Gly	Leu	Gln	Arg	Ala	Lys	Asn	Asn	Gly	Asn	Asn
				260					265					270		
	Met	Trp	Asn	Asn	Val	Asn	Asn	Tyr	Ile	Arg	Thr	Met	Lys	Leu	Asn	Ala
			275					280					285			
35	Leu	Asp	Thr	Val	Ala	Gln	Trp	Pro	Ile	Leu	Asp	Lys	Val	Thr	Tyr	Pro
		290					295					300				
	Leu	Asp	Thr	Thr	Leu	Gln	Thr	Arg	Gly	Ile	Phe	Ser	Asp	Leu	Ser	
	305					310				315					320	
	Gly	Arg	Gly	Gly	Thr	Gln	Ser	Asn	Tyr	Arg	Tyr	Asp	Tyr	Asp	Ala	Val
					325					330					335	
40	Gln	Gly	Tyr	Ala	Pro	Pro	Phe	Val	Gly	Phe	Asp	Thr	Lys	Leu	Asn	Val
				340					345					350		
	Val	Asn	Asp	Phe	Gly	Tyr	Lys	Asp	Leu	Thr	Ala	Ile	Gln	Thr	Phe	Thr
			355					360					365			
	Gly	Asp	Arg	Ile	Asp	Ser	Ile	Trp	Gln	Ser	Phe	Lys	Tyr	Asn	Ser	Gly
45		370					375					380				
	Glu	Pro	Phe	Leu	Thr	Asn	Leu	Gly	Asn	Gly	Lys	Pro	Gly	Asn	Asn	Pro
	385					390					395					400
	Val	Ile	Pro	Asn	Ser	Arg	Asp	Asn	Pro	Ile	Ile	Ser	Ala	Lys	Gly	Ser
				405						410					415	
	Arg	Pro	Ser	Ala	Asn	Tyr	Val	Gly	Met	Asn	Phe	Gln	Arg	Ala	Asn	Lys
50				420					425					430		
	Thr	Val	Val	Ser	Asn	Gly	Tyr	Val	Ile	Pro	Asn	Asp	Asn	Tyr	Thr	Val
			435					440						445		
	Pro	Ala	Gly	His	Lys	Leu	Gly	Trp	Ile	Ser	Ala	Leu	His	Asp	Glu	Leu
		450					455					460				
55	Asp	Asn	Ala	Asn	Asn	Ala	Asp	Leu	Val	Val	Ser	Val	Trp	Val	Lys	Asn
	465					470					475					480

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	Asp	Ile	Phe	Gln	Glu	Asn	Ile	Ile	Gly	Ser	Ile	Lys	Thr	Val	Thr	Thr
					485					490					495	
	Asp	Asp	Gly	Thr	Thr	Glu	Asn	Arg	Gln	Gln	Ile	Ile	Gly	Ile	Pro	Ala
			500						505					510		
5	Asp	Lys	His	Met	Thr	Arg	Ser	Thr	Lys	Arg	Met	Glu	Leu	Glu	Phe	Ile
		515						520					525			
	Asn	Gly	Thr	Asn	Gly	Ser	Met	Ser	Leu	Ser	Ser	Thr	Asn	Asp	Gln	Leu
		530					535					540				
	Tyr	Tyr	Thr	Ile	Asn	Pro	Ile	Val	Ser	Gln	Arg	Tyr	Gln	Ile	Arg	Tyr
10		545				550				555					560	
	Arg	Val	Ala	Thr	Thr	Ser	Ala	Glu	Ser	Leu	Asp	Leu	Trp	Ile	Asp	Gly
				565					570						575	
	Tyr	Lys	Arg	Gly	Thr	Thr	Pro	Leu	Pro	Asn	Thr	Ser	Ser	Thr	Ser	Thr
				580					585					590		
	Gln	Thr	Gln	Lys	Val	Ile	Ile	Gln	Gly	Leu	Gln	Gly	Lys	Tyr	Gln	Leu
15			595					600					605			
	Ile	Asn	Gly	Pro	Thr	Leu	Asp	Leu	Thr	Ala	Gly	Ser	His	Thr	Phe	Gly
		610					615					620				
	Ile	Met	Leu	Thr	Ala	Asn	Ala	Ser	Gln	Asn	Val	Phe	Ile	Asp	Arg	Ile
		625				630				635					640	
20	Glu	Phe	Val	Pro	Ile	Ala	Thr	Thr	Glu	Pro	Val	Thr	Ile	Pro	Asn	Thr
				645					650						655	
	Pro	Ile	Lys	Thr	Tyr	Thr	Asn	Pro	Pro	Asn	Pro	Gln	Gln	Val	Leu	Trp
			660					665						670		
	Thr	Ala	Gln	Pro	Gly	Ile	Leu	Gly	Asp	Ile	Val	Asn	Tyr	His	Ile	Asn
			675				680						685			
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		690														

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30 <212> PRT

<213> Bacillus thuringiensis

<400> 7

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	Ser	Asn	Pro	Glu	Val	Glu	Val	Leu	Gly	Gly	Glu	Arg	Ile	Glu	Thr	Gly
				20					25					30		
40	Tyr	Thr	Pro	Ile	Asp	Ile	Ser	Leu	Ser	Leu	Thr	Gln	Phe	Leu	Leu	Ser
			35					40					45			
	Glu	Phe	Val	Pro	Gly	Ala	Gly	Phe	Val	Leu	Gly	Leu	Val	Asp	Ile	Ile
		50					55					60				
	Trp	Gly	Ile	Phe	Gly	Pro	Ser	Gln	Trp	Asp	Ala	Phe	Leu	Val	Gln	Ile
		65			70					75					80	
45	Glu	Gln	Leu	Ile	Asn	Gln	Arg	Ile	Glu	Glu	Phe	Ala	Arg	Asn	Gln	Ala
				85					90						95	
	Ile	Ser	Arg	Leu	Glu	Gly	Leu	Ser	Asn	Leu	Tyr	Gln	Ile	Tyr	Ala	Glu
			100						105					110		
	Ser	Phe	Arg	Glu	Trp	Glu	Ala	Asp	Pro	Thr	Asn	Pro	Ala	Leu	Arg	Glu
			115					120					125			
50	Glu	Met	Arg	Ile	Gln	Phe	Asn	Asp	Met	Asn	Ser	Ala	Leu	Thr	Thr	Ala
		130					135					140				
	Ile	Pro	Leu	Phe	Ala	Val	Gln	Asn	Tyr	Gln	Val	Pro	Leu	Leu	Ser	Val
				145		150				155					160	
	Tyr	Val	Gln	Ala	Ala	Asn	Leu	His	Leu	Ser	Val	Leu	Arg	Asp	Val	Ser
				165					170					175		
55	Val	Phe	Gly	Gln	Arg	Trp	Gly	Phe	Asp	Ala	Ala	Thr	Ile	Asn	Ser	Arg
				180					185					190		

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Tyr Asn Asp Leu Thr Arg Leu Ile Gly Asn Tyr Thr Asp Tyr Ala Val
 195 200 205
 Arg Trp Tyr Asn Thr Gly Leu Glu Arg Val Trp Gly Pro Asp Ser Arg
 210 215 220
 5 Asp Trp Val Arg Tyr Asn Gln Phe Arg Arg Glu Leu Thr Leu Thr Val
 225 230 235 240
 Leu Asp Ile Val Ala Leu Phe Pro Asn Tyr Asp Ser Arg Arg Tyr Pro
 245 250 255
 Ile Arg Thr Val Ser Gln Leu Thr Arg Glu Ile Tyr Thr Asn Pro Val
 260 265 270
 10 Leu Glu Asn Phe Asp Gly Ser Phe Arg Gly Ser Ala Gln Gly Ile Glu
 275 280 285
 Ser Ile Arg Ser Pro His Leu Met Asp Ile Leu Asn Ser Ile Thr
 290 295 300
 15 Ile Tyr Thr Asp Ala His Arg Gly Tyr Tyr Tyr Trp Ser Gly His Gln
 305 310 315 320
 Ile Met Ala Ser Pro Val Gly Phe Ser Gly Pro Glu Phe Thr Phe Pro
 325 330 335
 Leu Tyr Gly Thr Met Gly Asn Ala Ala Pro Gln Gln Arg Ile Val Ala
 340 345 350
 20 Gln Leu Gly Gln Gly Val Tyr Arg Thr Leu Ser Ser Thr Leu Tyr Arg
 355 360 365
 Arg Pro Phe Asn Ile Gly Ile Asn Asn Gln Gln Leu Ser Val Leu Asp
 370 375 380
 Gly Thr Glu Phe Ala Tyr Gly Thr Ser Ser Asn Leu Pro Ser Ala Val
 385 390 395 400
 25 Tyr Arg Lys Ser Gly Thr Val Asp Ser Leu Asp Glu Ile Pro Pro Gln
 405 410 415
 Asn Asn Asn Val Pro Pro Arg Gln Gly Phe Ser His Arg Leu Ser His
 420 425 430
 Val Ser Met Phe Arg Ser Gly Phe Ser Asn Ser Ser Val Ser Ile Ile
 435 440 445
 30 Arg Ala Pro Met Phe Ser Trp Ile His Arg Ser Ala Glu Phe Asn Asn
 450 455 460
 Ile Ile Ala Ser Asp Ser Ile Thr Gln Ile Pro Ala Val Lys Gly Asn
 465 470 475 480
 35 Phe Leu Phe Asn Gly Ser Val Ile Ser Gly Pro Gly Phe Thr Gly Gly
 485 490 495
 Asp Leu Val Arg Leu Asn Ser Ser Gly Asn Asn Ile Gln Asn Arg Gly
 500 505 510
 Tyr Ile Glu Val Pro Ile His Phe Pro Ser Thr Ser Thr Arg Tyr Arg
 515 520 525
 40 Val Arg Val Arg Tyr Ala Ser Val Thr Pro Ile His Leu Asn Val Asn
 530 535 540
 Trp Gly Asn Ser Ser Ile Phe Ser Asn Thr Val Pro Ala Thr Ala Thr
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 Ser Leu Asp Asn Leu Gln Ser Ser Asp Phe Gly Tyr Phe Glu Ser Ala
 565 570 575
 45 Asn Ala Phe Thr Ser Ser Leu Gly Asn Ile Val Gly Val Arg Asn Phe
 580 585 590
 Ser Gly Thr Ala Gly Val Ile Ile Asp Arg Phe Glu Phe Ile Pro Val
 595 600 605
 50 Thr Ala Thr Leu Glu Ala Glu Tyr Asn Leu Glu Arg Ala Gln Lys Ala
 610 615 620
 Val Asn Ala Leu Phe Thr Ser Thr Asn Gln Leu Gly Leu Lys Thr Asn
 625 630 635 640
 Val Thr Asp Tyr His Ile Asp Gln Val Ser Asn Leu Val Thr Tyr Leu
 645 650 655
 55 Ser Asp Glu Phe Cys Leu Asp Glu Lys Arg Glu Leu Ser Glu Lys Val
 660 665 670

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	Lys	His	Ala	Lys	Arg	Leu	Ser	Asp	Glu	Arg	Asn	Leu	Leu	Gln	Asp	Ser
			675					680					685			
	Asn	Phe	Lys	Asp	Ile	Asn	Arg	Gln	Pro	Glu	Arg	Gly	Trp	Gly	Gly	Ser
		690				695						700				
5	Thr	Gly	Ile	Thr	Ile	Gln	Gly	Gly	Asp	Asp	Val	Phe	Lys	Glu	Asn	Tyr
	705					710					715					720
	Val	Thr	Leu	Ser	Gly	Thr	Phe	Asp	Glu	Cys	Tyr	Pro	Thr	Tyr	Leu	Tyr
					725					730					735	
	Gln	Lys	Ile	Asp	Glu	Ser	Lys	Leu	Lys	Ala	Phe	Thr	Arg	Tyr	Gln	Leu
10				740					745					750		
	Arg	Gly	Tyr	Ile	Glu	Asp	Ser	Gln	Asp	Leu	Glu	Ile	Tyr	Leu	Ile	Arg
		755						760					765			
	Tyr	Asn	Ala	Lys	His	Glu	Thr	Val	Asn	Val	Pro	Gly	Thr	Gly	Ser	Leu
		770					775					780				
	Trp	Pro	Leu	Ser	Ala	Gln	Ser	Pro	Ile	Gly	Lys	Cys	Gly	Glu	Pro	Asn
15		785				790					795					800
	Arg	Cys	Ala	Pro	His	Leu	Glu	Trp	Asn	Pro	Asp	Leu	Asp	Cys	Ser	Cys
					805					810					815	
	Arg	Asp	Gly	Glu	Lys	Cys	Ala	His	His	Ser	His	His	Phe	Ser	Leu	Asp
				820					825					830		
20	Ile	Asp	Val	Gly	Cys	Thr	Asp	Leu	Asn	Glu	Asp	Leu	Gly	Val	Trp	Val
			835					840					845			
	Ile	Phe	Lys	Ile	Lys	Thr	Gln	Asp	Gly	His	Ala	Arg	Leu	Gly	Asn	Leu
		850					855					860				
	Glu	Phe	Leu	Glu	Glu	Lys	Pro	Leu	Val	Gly	Glu	Ala	Leu	Ala	Arg	Val
25		865				870					875					880
	Lys	Arg	Ala	Glu	Lys	Lys	Trp	Arg	Asp	Lys	Arg	Glu	Lys	Leu	Glu	Trp
					885					890					895	
	Glu	Thr	Asn	Ile	Val	Tyr	Lys	Glu	Ala	Lys	Glu	Ser	Val	Asp	Ala	Leu
			900						905					910		
	Phe	Val	Asn	Ser	Gln	Tyr	Asp	Gln	Leu	Gln	Ala	Asp	Thr	Asn	Ile	Ala
30			915					920					925			
	Met	Ile	His	Ala	Ala	Asp	Lys	Arg	Val	His	Ser	Ile	Arg	Glu	Ala	Tyr
		930					935					940				
	Leu	Pro	Glu	Leu	Ser	Val	Ile	Pro	Gly	Val	Asn	Ala	Ala	Ile	Phe	Glu
		945				950					955					960
35	Glu	Leu	Glu	Gly	Arg	Ile	Phe	Thr	Ala	Phe	Ser	Leu	Tyr	Asp	Ala	Arg
					965					970					975	
	Asn	Val	Ile	Lys	Asn	Gly	Asp	Phe	Asn	Asn	Gly	Leu	Ser	Cys	Trp	Asn
				980					985					990		
	Val	Lys	Gly	His	Val	Asp	Val	Glu	Gln	Asn	Asn	Gln	Arg	Ser	Val	
		995						1000					1005			
40	Leu	Val	Val	Pro	Glu	Trp	Glu	Ala	Glu	Val	Ser	Gln	Glu	Val	Arg	Val
		1010					1015					1020				
	Cys	Pro	Gly	Arg	Gly	Tyr	Ile	Leu	Arg	Val	Thr	Ala	Tyr	Lys	Glu	Gly
		1025				1030					1035					1040
45	Tyr	Gly	Glu	Gly	Cys	Val	Thr	Ile	His	Glu	Ile	Glu	Asn	Asn	Thr	Asp
					1045					1050					1055	
	Glu	Leu	Lys	Phe	Ser	Asn	Cys	Val	Glu	Glu	Glu	Ile	Tyr	Pro	Asn	Asn
				1060					1065					1070		
	Thr	Val	Thr	Cys	Asn	Asp	Tyr	Thr	Val	Asn	Gln	Glu	Glu	Tyr	Gly	Gly
			1075					1080					1085			
50	Ala	Tyr	Thr	Ser	Arg	Asn	Arg	Gly	Tyr	Asn	Glu	Ala	Pro	Ser	Val	Pro
		1090					1095					1100				
	Ala	Asp	Tyr	Ala	Ser	Val	Tyr	Glu	Glu	Lys	Ser	Tyr	Thr	Asp	Gly	Arg
		1105				1110					1115					1120
	Arg	Glu	Asn	Pro	Cys	Glu	Phe	Asn	Arg	Gly	Tyr	Arg	Asp	Tyr	Thr	Pro
				1125						1130					1135	
55	Leu	Pro	Val	Gly	Tyr	Val	Thr	Lys	Glu	Leu	Glu	Tyr	Phe	Pro	Glu	Thr
				1140					1145					1150		

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Asp Lys Val Trp Ile Glu Ile Gly Glu Thr Glu Gly Thr Phe Ile Val
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 Asp Ser Val Glu Leu Leu Leu Met Glu Glu
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<400> 8

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 35 40 45
 Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly Arg Ile Leu Gly Val
 50 55 60
 Leu Gly Val Pro Phe Ala Gly Gln Leu Ala Ser Phe Tyr Ser Phe Leu
 65 70 75 80
 Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Gln Trp Glu Ile Phe Leu
 85 90 95
 Glu His Val Glu Gln Leu Ile Asn Gln Gln Ile Thr Glu Asn Ala Arg
 100 105 110
 Asn Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly Asp Ser Phe Arg Ala
 115 120 125
 Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn Arg Asp Ala Arg
 130 135 140
 Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala Leu Glu Leu Asp Phe
 145 150 155 160
 Leu Asn Ala Met Pro Leu Phe Ala Ile Arg Asn Gln Glu Val Pro Leu
 165 170 175
 Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His Leu Leu Leu Leu Arg
 180 185 190
 Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu Thr Ser Gln Glu Ile
 195 200 205
 Gln Arg Tyr Tyr Glu Arg Gln Val Glu Arg Thr Arg Asp Tyr Ser Asp
 210 215 220
 Tyr Cys Val Glu Trp Tyr Asn Thr Gly Leu Asn Ser Leu Arg Gly Thr
 225 230 235 240
 Asn Ala Ala Ser Trp Val Arg Tyr Asn Gln Phe Arg Arg Asp Leu Thr
 245 250 255
 Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro Ser Tyr Asp Thr Arg
 260 265 270
 Thr Tyr Pro Ile Asn Thr Ser Ala Gln Leu Thr Arg Glu Val Tyr Thr
 275 280 285
 Asp Ala Ile Gly Ala Thr Gly Val Asn Met Ala Ser Met Asn Trp Tyr
 290 295 300
 Asn Asn Asn Ala Pro Ser Phe Ser Ala Ile Glu Ala Ala Ala Ile Arg
 305 310 315 320
 Ser Pro His Leu Leu Asp Phe Leu Glu Gln Leu Thr Ile Phe Ser Ala
 325 330 335
 Ser Ser Arg Trp Ser Asn Thr Arg His Met Thr Tyr Trp Arg Gly His
 340 345 350
 Thr Ile Gln Ser Arg Pro Ile Gly Gly Gly Leu Asn Thr Ser Thr His
 355 360 365
 Gly Ala Thr Asn Thr Ser Ile Asn Pro Val Thr Leu Arg Phe Ala Ser
 370 375 380

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	Arg	Asp	Val	Tyr	Arg	Thr	Glu	Ser	Tyr	Ala	Gly	Val	Leu	Leu	Trp	Gly
	385					390					395					400
	Ile	Tyr	Leu	Glu	Pro	Ile	His	Gly	Val	Pro	Thr	Val	Arg	Phe	Asn	Phe
					405					410					415	
5	Thr	Asn	Pro	Gln	Asn	Ile	Ser	Asp	Arg	Gly	Thr	Ala	Asn	Tyr	Ser	Gln
				420					425					430		
	Pro	Tyr	Glu	Ser	Pro	Gly	Leu	Gln	Leu	Lys	Asp	Ser	Glu	Thr	Glu	Leu
			435				440						445			
	Pro	Pro	Glu	Thr	Thr	Glu	Arg	Pro	Asn	Tyr	Glu	Ser	Tyr	Ser	His	Arg
10		450				455					460					
	Leu	Ser	His	Ile	Gly	Ile	Ile	Leu	Gln	Ser	Arg	Val	Asn	Val	Pro	Val
	465				470						475				480	
	Tyr	Ser	Trp	Thr	His	Arg	Ser	Ala	Asp	Arg	Thr	Asn	Thr	Ile	Gly	Pro
					485					490					495	
	Asn	Arg	Ile	Thr	Gln	Ile	Pro	Met	Val	Lys	Ala	Ser	Glu	Leu	Pro	Gln
15				500					505					510		
	Gly	Thr	Thr	Val	Val	Arg	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asp	Ile	Leu
			515					520					525			
	Arg	Arg	Thr	Asn	Thr	Gly	Gly	Phe	Gly	Pro	Ile	Arg	Val	Thr	Val	Asn
		530				535						540				
20	Gly	Pro	Leu	Thr	Gln	Arg	Tyr	Arg	Ile	Gly	Phe	Arg	Tyr	Ala	Ser	Thr
	545				550						555					560
	Val	Asp	Phe	Asp	Phe	Phe	Val	Ser	Arg	Gly	Gly	Thr	Thr	Val	Asn	Asn
					565					570					575	
	Phe	Arg	Phe	Leu	Arg	Thr	Met	Asn	Ser	Gly	Asp	Glu	Leu	Lys	Tyr	Gly
25				580					585					590		
	Asn	Phe	Val	Arg	Arg	Ala	Phe	Thr	Thr	Pro	Phe	Thr	Phe	Thr	Gln	Ile
			595					600					605			
	Gln	Asp	Ile	Ile	Arg	Thr	Ser	Ile	Gln	Gly	Leu	Ser	Gly	Asn	Gly	Glu
		610				615						620				
30	Val	Tyr	Ile	Asp	Lys	Ile	Glu	Ile	Ile	Pro	Val	Thr	Ala	Thr	Phe	Glu
	625				630						635					640
	Ala	Glu	Tyr	Asp	Leu	Glu	Arg	Ala	Gln	Glu	Ala	Val	Asn	Ala	Leu	Phe
					645					650					655	
	Thr	Asn	Thr	Asn	Pro	Arg	Arg	Leu	Lys	Thr	Asp	Val	Thr	Asp	Tyr	His
				660					665					670		
35	Ile	Asp	Gln	Val	Ser	Asn	Leu	Val	Ala	Cys	Leu	Ser	Asp	Glu	Phe	Cys
			675				680						685			
	Leu	Asp	Glu	Lys	Arg	Glu	Leu	Leu	Glu	Lys	Val	Lys	Tyr	Ala	Lys	Arg
		690				695						700				
	Leu	Ser	Asp	Glu	Arg	Asn	Leu	Leu	Gln	Asp	Pro	Asn	Phe	Thr	Ser	Ile
40		705				710					715					720
	Asn	Lys	Gln	Pro	Asp	Phe	Ile	Ser	Thr	Asn	Glu	Gln	Ser	Asn	Phe	Thr
				725						730					735	
	Ser	Ile	His	Glu	Gln	Ser	Glu	His	Gly	Trp	Trp	Gly	Ser	Glu	Asn	Ile
				740					745					750		
45	Thr	Ile	Gln	Glu	Gly	Asn	Asp	Val	Phe	Lys	Glu	Asn	Tyr	Val	Thr	Leu
			755					760					765			
	Pro	Gly	Thr	Phe	Asn	Glu	Cys	Tyr	Pro	Thr	Tyr	Leu	Tyr	Gln	Lys	Ile
		770				775						780				
	Gly	Glu	Ser	Glu	Leu	Lys	Ala	Tyr	Thr	Arg	Tyr	Gln	Leu	Arg	Gly	Tyr
					790						795					800
50	Ile	Glu	Asp	Ser	Gln	Asp	Leu	Glu	Ile	Tyr	Leu	Ile	Arg	Tyr	Asn	Ala
					805					810					815	
	Lys	His	Glu	Thr	Leu	Asp	Val	Pro	Gly	Thr	Glu	Ser	Leu	Trp	Pro	Leu
				820					825					830		
	Ser	Val	Glu	Ser	Pro	Ile	Gly	Arg	Cys	Gly	Glu	Pro	Asn	Arg	Cys	Ala
			835					840					845			
55	Pro	His	Phe	Glu	Trp	Asn	Pro	Asp	Leu	Asp	Cys	Ser	Cys	Arg	Asp	Gly
		850					855						860			

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Glu Lys Cys Ala His His Ser His His Phe Ser Leu Asp Ile Asp Val
 865 870 875 880
 Gly Cys Thr Asp Leu His Glu Asn Leu Gly Val Trp Val Val Phe Lys
 885 890 895
 5 Ile Lys Thr Gln Glu Gly His Ala Arg Leu Gly Asn Leu Glu Phe Ile
 900 905 910
 Glu Glu Lys Pro Leu Leu Gly Glu Ala Leu Ser Arg Val Lys Arg Ala
 915 920 925
 10 Glu Lys Lys Trp Arg Asp Lys Arg Glu Lys Leu Gln Leu Glu Thr Lys
 930 935 940
 Arg Val Tyr Thr Glu Ala Lys Glu Ala Val Asp Ala Leu Phe Val Asp
 945 950 955 960
 Ser Gln Tyr Asp Arg Leu Gln Ala Asp Thr Asn Ile Gly Met Ile His
 965 970 975
 15 Ala Ala Asp Lys Leu Val His Arg Ile Arg Glu Ala Tyr Leu Ser Glu
 980 985 990
 Leu Pro Val Ile Pro Gly Val Asn Ala Glu Ile Phe Glu Glu Leu Glu
 995 1000 1005
 Gly His Ile Ile Thr Ala Ile Ser Leu Tyr Asp Ala Arg Asn Val Val
 1010 1015 1020
 20 Lys Asn Gly Asp Phe Asn Asn Gly Leu Thr Cys Trp Asn Val Lys Gly
 1025 1030 1035 1040
 His Val Asp Val Gln Gln Ser His His Arg Ser Asp Leu Val Ile Pro
 1045 1050 1055
 25 Glu Trp Glu Ala Glu Val Ser Gln Ala Val Arg Val Cys Pro Gly Cys
 1060 1065 1070
 Gly Tyr Ile Leu Arg Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly
 1075 1080 1085
 Cys Val Thr Ile His Glu Ile Glu Asn Asn Thr Asp Glu Leu Lys Phe
 1090 1095 1100
 30 Lys Asn Arg Glu Glu Glu Glu Val Tyr Pro Thr Asp Thr Gly Thr Cys
 1105 1110 1115 1120
 Asn Asp Tyr Thr Ala His Gln Gly Thr Ala Gly Cys Ala Asp Ala Cys
 1125 1130 1135
 Asn Ser Arg Asn Ala Gly Tyr Glu Asp Ala Tyr Glu Val Asp Thr Thr
 1140 1145 1150
 35 Ala Ser Val Asn Tyr Lys Pro Thr Tyr Glu Glu Glu Thr Tyr Thr Asp
 1155 1160 1165
 Val Arg Arg Asp Asn His Cys Glu Tyr Asp Arg Gly Tyr Val Asn Tyr
 1170 1175 1180
 40 Pro Pro Val Pro Ala Gly Tyr Val Thr Lys Glu Leu Glu Tyr Phe Pro
 1185 1190 1195 1200
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<210> 9

<211> 1189

<212> PRT

50 <213> Bacillus thuringiensis

<400> 9

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	Met	Glu	Glu	Asn	Asn	Gln	Asn	Gln	Cys	Ile	Pro	Tyr	Asn	Cys	Leu	Ser
	1				5				10						15	
	Asn	Pro	Glu	Glu	Val	Leu	Leu	Asp	Gly	Glu	Arg	Ile	Ser	Thr	Gly	Asn
			20					25					30			
5	Ser	Ser	Ile	Asp	Ile	Ser	Leu	Ser	Leu	Val	Gln	Phe	Leu	Val	Ser	Asn
			35				40						45			

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	Phe	Val	Pro	Gly	Gly	Gly	Phe	Leu	Val	Gly	Leu	Ile	Asp	Phe	Val	Trp
	50						55					60				
	Gly	Ile	Val	Gly	Pro	Ser	Gln	Trp	Asp	Ala	Phe	Leu	Val	Gln	Ile	Glu
	65					70				75						80
5	Gln	Leu	Ile	Asn	Glu	Arg	Ile	Ala	Glu	Phe	Ala	Arg	Asn	Ala	Ala	Ile
				85						90					95	
	Ala	Asn	Leu	Glu	Gly	Leu	Gly	Asn	Asn	Phe	Asn	Ile	Tyr	Val	Glu	Ala
				100					105					110		
	Phe	Lys	Glu	Trp	Glu	Glu	Asp	Pro	Asn	Asn	Pro	Ala	Thr	Arg	Thr	Arg
10			115				120						125			
	Val	Ile	Asp	Arg	Phe	Arg	Ile	Leu	Asp	Gly	Leu	Leu	Glu	Arg	Asp	Ile
	130						135						140			
	Pro	Ser	Phe	Arg	Ile	Ser	Gly	Phe	Glu	Val	Pro	Leu	Leu	Ser	Val	Tyr
	145					150					155					160
15	Ala	Gln	Ala	Ala	Asn	Leu	His	Leu	Ala	Ile	Leu	Arg	Asp	Ser	Val	Ile
				165						170					175	
	Phe	Gly	Glu	Arg	Trp	Gly	Leu	Thr	Thr	Ile	Asn	Val	Asn	Glu	Asn	Tyr
				180					185					190		
	Asn	Arg	Leu	Ile	Arg	His	Ile	Asp	Glu	Tyr	Ala	Asp	His	Cys	Ala	Asn
			195					200					205			
20	Thr	Tyr	Asn	Arg	Gly	Leu	Asn	Asn	Leu	Pro	Lys	Ser	Thr	Tyr	Gln	Asp
		210					215					220				
	Trp	Ile	Thr	Tyr	Asn	Arg	Leu	Arg	Arg	Asp	Leu	Thr	Leu	Thr	Val	Leu
	225				230					235						240
	Asp	Ile	Ala	Ala	Phe	Phe	Pro	Asn	Tyr	Asp	Asn	Arg	Arg	Tyr	Pro	Ile
25				245						250					255	
	Gln	Pro	Val	Gly	Gln	Leu	Thr	Arg	Glu	Val	Tyr	Thr	Asp	Pro	Leu	Ile
				260					265					270		
	Asn	Phe	Asn	Pro	Gln	Leu	Gln	Ser	Val	Ala	Gln	Leu	Pro	Thr	Phe	Asn
			275					280					285			
30	Val	Met	Glu	Ser	Ser	Ala	Ile	Arg	Asn	Pro	His	Leu	Phe	Asp	Ile	Leu
		290					295					300				
	Asn	Asn	Leu	Thr	Ile	Phe	Thr	Asp	Trp	Phe	Ser	Val	Gly	Arg	Asn	Phe
	305				310					315						320
	Tyr	Trp	Gly	Gly	His	Arg	Val	Ile	Ser	Ser	Leu	Ile	Gly	Gly	Gly	Asn
				325					330					335		
35	Ile	Thr	Ser	Pro	Ile	Tyr	Gly	Arg	Glu	Ala	Asn	Gln	Glu	Pro	Pro	Arg
				340					345					350		
	Ser	Phe	Thr	Phe	Asn	Gly	Pro	Val	Phe	Arg	Thr	Leu	Ser	Asn	Pro	Thr
			355					360					365			
	Leu	Arg	Leu	Leu	Gln	Gln	Pro	Trp	Pro	Ala	Pro	Pro	Phe	Asn	Leu	Arg
40			370				375					380				
	Gly	Val	Glu	Gly	Val	Glu	Phe	Ser	Thr	Pro	Thr	Asn	Ser	Phe	Thr	Tyr
	385					390					395					400
	Arg	Gly	Arg	Gly	Thr	Val	Asp	Ser	Leu	Thr	Glu	Leu	Pro	Pro	Glu	Asp
				405						410					415	
45	Asn	Ser	Val	Pro	Pro	Arg	Glu	Gly	Tyr	Ser	His	Arg	Leu	Cys	His	Ala
			420						425					430		
	Thr	Phe	Val	Gln	Arg	Ser	Gly	Thr	Pro	Phe	Leu	Thr	Thr	Gly	Val	Val
			435					440					445			
	Phe	Ser	Trp	Thr	His	Arg	Ser	Ala	Thr	Leu	Thr	Asn	Thr	Ile	Asp	Pro
			450				455					460				
50	Glu	Arg	Ile	Asn	Gln	Ile	Pro	Leu	Val	Lys	Gly	Phe	Arg	Val	Trp	Gly
	465					470					475					480
	Gly	Thr	Ser	Val	Ile	Thr	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asp	Ile	Leu
				485						490					495	
	Arg	Arg	Asn	Thr	Phe	Gly	Asp	Phe	Val	Ser	Leu	Gln	Val	Asn	Ile	Asn
			500					505					510			
55	Ser	Pro	Ile	Thr	Gln	Arg	Tyr	Arg	Leu	Arg	Phe	Arg	Tyr	Ala	Ser	Ser
			515					520					525			

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	Arg	Asp	Ala	Arg	Val	Ile	Val	Leu	Thr	Gly	Ala	Ala	Ser	Thr	Gly	Val
	530						535					540				
	Gly	Gly	Gln	Val	Ser	Val	Asn	Met	Pro	Leu	Gln	Lys	Thr	Met	Glu	Ile
	545					550					555					560
5	Gly	Glu	Asn	Leu	Thr	Ser	Arg	Thr	Phe	Arg	Tyr	Thr	Asp	Phe	Ser	Asn
					565					570					575	
	Pro	Phe	Ser	Phe	Arg	Ala	Asn	Pro	Asp	Ile	Ile	Gly	Ile	Ser	Glu	Gln
				580					585					590		
	Pro	Leu	Phe	Gly	Ala	Gly	Ser	Ile	Ser	Ser	Gly	Glu	Leu	Tyr	Ile	Asp
10			595				600						605			
	Lys	Ile	Glu	Ile	Ile	Leu	Ala	Asp	Ala	Thr	Phe	Glu	Ala	Glu	Ser	Asp
	610						615					620				
	Leu	Glu	Arg	Ala	Gln	Lys	Ala	Val	Asn	Ala	Leu	Phe	Thr	Ser	Ser	Asn
	625					630					635					640
15	Gln	Ile	Gly	Leu	Lys	Thr	Asp	Val	Thr	Asp	Tyr	His	Ile	Asp	Gln	Val
					645					650					655	
	Ser	Asn	Leu	Val	Asp	Cys	Leu	Ser	Asp	Glu	Phe	Cys	Leu	Asp	Glu	Lys
				660					665					670		
	Arg	Glu	Leu	Ser	Glu	Lys	Val	Lys	His	Ala	Lys	Arg	Leu	Ser	Asp	Glu
			675					680					685			
20	Arg	Asn	Leu	Leu	Gln	Asp	Pro	Asn	Phe	Arg	Gly	Ile	Asn	Arg	Gln	Pro
							695					700				
	Asp	Arg	Gly	Trp	Arg	Gly	Ser	Thr	Asp	Ile	Thr	Ile	Gln	Gly	Gly	Asp
	705					710					715					720
	Asp	Val	Phe	Lys	Glu	Asn	Tyr	Val	Thr	Leu	Pro	Gly	Thr	Val	Asp	Glu
25					725					730					735	
	Cys	Tyr	Pro	Thr	Tyr	Leu	Tyr	Gln	Lys	Ile	Asp	Glu	Ser	Lys	Leu	Lys
				740					745					750		
	Ala	Tyr	Thr	Arg	Tyr	Glu	Leu	Arg	Gly	Tyr	Ile	Glu	Asp	Ser	Gln	Asp
			755					760					765			
30	Leu	Glu	Ile	Tyr	Leu	Ile	Arg	Tyr	Asn	Ala	Lys	His	Glu	Ile	Val	Asn
							775					780				
	Val	Pro	Gly	Thr	Gly	Ser	Leu	Trp	Pro	Leu	Ser	Ala	Gln	Ser	Pro	Ile
	785					790					795					800
	Gly	Lys	Cys	Gly	Glu	Pro	Asn	Arg	Cys	Ala	Pro	His	Leu	Glu	Trp	Asn
					805					810					815	
35	Pro	Asp	Leu	Asp	Cys	Ser	Cys	Arg	Asp	Gly	Glu	Lys	Cys	Ala	His	His
				820					825					830		
	Ser	His	His	Phe	Thr	Leu	Asp	Ile	Asp	Val	Gly	Cys	Thr	Asp	Leu	Asn
				835				840					845			
	Glu	Asp	Leu	Gly	Val	Trp	Val	Ile	Phe	Lys	Ile	Lys	Thr	Gln	Asp	Gly
				850			855					860				
40	His	Ala	Arg	Leu	Gly	Asn	Leu	Glu	Phe	Leu	Glu	Glu	Lys	Pro	Leu	Leu
						870					875					880
	Gly	Glu	Ala	Leu	Ala	Arg	Val	Lys	Arg	Ala	Glu	Lys	Lys	Trp	Arg	Asp
					885					890					895	
45	Lys	Arg	Glu	Lys	Leu	Gln	Leu	Glu	Thr	Asn	Ile	Val	Tyr	Lys	Glu	Ala
				900					905					910		
	Lys	Glu	Ser	Val	Asp	Ala	Leu	Phe	Val	Asn	Ser	Gln	Tyr	Asp	Arg	Leu
				915					920					925		
	Gln	Val	Asp	Thr	Asn	Ile	Ala	Met	Ile	His	Ala	Ala	Asp	Lys	Arg	Val
				930			935					940				
50	His	Arg	Ile	Arg	Glu	Ala	Tyr	Leu	Pro	Glu	Leu	Ser	Val	Ile	Pro	Gly
	945					950					955					960
	Val	Asn	Ala	Ala	Ile	Phe	Glu	Glu	Leu	Glu	Gly	Arg	Ile	Phe	Thr	Ala
					965					970					975	
	Tyr	Ser	Leu	Tyr	Asp	Ala	Arg	Asn	Val	Ile	Lys	Asn	Gly	Asp	Phe	Asn
				980				985						990		
55	Asn	Gly	Leu	Leu	Cys	Trp	Asn	Val	Lys	Gly	His	Val	Asp	Val	Glu	Glu
			995					1000					1005			

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5 Gln Asn Asn His Arg Ser Val Leu Val Ile Pro Glu Trp Glu Ala Glu
 1010 1015 1020
 Val Ser Gln Glu Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg
 1025 1030 1035 1040
 Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile His
 1045 1050 1055
 Glu Ile Glu Asp Asn Thr Asp Glu Leu Lys Phe Ser Asn Cys Val Glu
 1060 1065 1070
 10 Glu Glu Val Tyr Pro Asn Asn Thr Val Thr Cys Asn Asn Tyr Thr Gly
 1075 1080 1085
 Thr Gln Glu Glu Tyr Glu Gly Thr Tyr Thr Ser Arg Asn Gln Gly Tyr
 1090 1095 1100
 Asp Glu Ala Tyr Gly Asn Asn Pro Ser Val Pro Ala Asp Tyr Ala Ser
 1105 1110 1115 1120
 15 Val Tyr Glu Glu Lys Ser Tyr Thr Asp Gly Arg Arg Glu Asn Pro Cys
 1125 1130 1135
 Glu Ser Asn Arg Gly Tyr Gly Asp Tyr Thr Pro Leu Pro Ala Gly Tyr
 1140 1145 1150
 Val Thr Lys Asp Leu Glu Tyr Phe Pro Glu Thr Asp Lys Val Trp Ile
 1155 1160 1165
 Glu Ile Gly Glu Thr Glu Gly Thr Phe Ile Val Asp Ser Val Glu Leu
 1170 1175 1180
 Leu Leu Met Glu Glu
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	Ala	Tyr	Thr	Pro	Pro	Ser	Phe	Leu	Pro	Asp	Ala	Gly	Thr	Gln	Ala	Thr
				20					25					30		
5	Pro	Ala	Asp	Leu	Thr	Ala	Tyr	Glu	Gln	Leu	Leu	Lys	Asn	Leu	Glu	Lys
			35					40					45			
	Gly	Ile	Asn	Ala	Gly	Thr	Tyr	Ser	Lys	Ala	Ile	Ala	Asp	Val	Leu	Lys
		50					55					60				
	Gly	Ile	Phe	Ile	Asp	Asp	Thr	Ile	Asn	Tyr	Gln	Thr	Tyr	Val	Asn	Ile
10	65					70					75				80	
	Gly	Leu	Ser	Leu	Ile	Thr	Leu	Ala	Val	Pro	Glu	Ile	Gly	Ile	Phe	Thr
					85					90					95	
	Pro	Phe	Ile	Gly	Leu	Phe	Phe	Ala	Ala	Leu	Asn	Lys	His	Asp	Ala	Pro
				100				105						110		
15	Pro	Pro	Pro	Asn	Ala	Lys	Asp	Ile	Phe	Glu	Ala	Met	Lys	Pro	Ala	Ile
			115				120						125			
	Gln	Glu	Met	Ile	Asp	Arg	Thr	Leu	Thr	Ala	Asp	Glu	Gln	Thr	Phe	Leu
		130				135						140				
	Asn	Gly	Glu	Ile	Ser	Gly	Leu	Gln	Asn	Leu	Ala	Ala	Arg	Tyr	Gln	Ser
20	145					150					155				160	
	Thr	Met	Asp	Asp	Ile	Gln	Ser	His	Gly	Gly	Phe	Asn	Lys	Val	Asp	Ser
				165						170					175	
	Gly	Leu	Ile	Lys	Lys	Phe	Thr	Asp	Glu	Val	Leu	Ser	Leu	Asn	Ser	Phe
			180					185						190		
25	Tyr	Thr	Asp	Arg	Leu	Pro	Val	Phe	Ile	Thr	Asp	Asn	Thr	Ala	Asp	Arg
			195				200						205			
	Thr	Leu	Leu	Gly	Leu	Pro	Tyr	Tyr	Ala	Ile	Leu	Ala	Ser	Met	His	Leu
		210				215						220				

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	Met	Leu	Leu	Arg	Asp	Ile	Ile	Thr	Lys	Gly	Pro	Thr	Trp	Asp	Ser	Lys
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	Ile	Asn	Phe	Thr	Pro	Asp	Ala	Ile	Asp	Ser	Phe	Lys	Thr	Asp	Ile	Lys
					245					250					255	
5	Asn	Asn	Ile	Lys	Leu	Tyr	Ser	Lys	Thr	Ile	Tyr	Asp	Val	Phe	Gln	Lys
				260					265					270		
	Gly	Leu	Ala	Ser	Tyr	Gly	Thr	Pro	Ser	Asp	Leu	Glu	Ser	Phe	Ala	Lys
			275					280					285			
	Lys	Gln	Lys	Tyr	Ile	Glu	Ile	Met	Thr	Thr	His	Cys	Leu	Asp	Phe	Ala
10		290					295					300				
	Arg	Leu	Phe	Pro	Thr	Phe	Asp	Pro	Asp	Leu	Tyr	Pro	Thr	Gly	Ser	Gly
	305					310					315					320
	Asp	Ile	Ser	Leu	Gln	Lys	Thr	Arg	Arg	Ile	Leu	Ser	Pro	Phe	Ile	Pro
					325					330					335	
	Ile	Arg	Thr	Ala	Asp	Gly	Leu	Thr	Leu	Asn	Asn	Thr	Ser	Ile	Asp	Thr
15				340				345						350		
	Ser	Asn	Trp	Pro	Asn	Tyr	Glu	Asn	Gly	Asn	Gly	Ala	Phe	Pro	Asn	Pro
			355					360					365			
	Lys	Glu	Arg	Ile	Leu	Lys	Gln	Phe	Lys	Leu	Tyr	Pro	Ser	Trp	Arg	Ala
		370					375					380				
20	Gly	Gln	Tyr	Gly	Gly	Leu	Leu	Gln	Pro	Tyr	Leu	Trp	Ala	Ile	Glu	Val
	385					390					395					400
	Gln	Asp	Ser	Val	Glu	Thr	Arg	Leu	Tyr	Gly	Gln	Leu	Pro	Ala	Val	Asp
				405						410					415	
	Pro	Gln	Ala	Gly	Pro	Asn	Tyr	Val	Ser	Ile	Asp	Ser	Ser	Asn	Pro	Ile
25				420					425					430		
	Ile	Gln	Ile	Asn	Met	Asp	Thr	Trp	Lys	Thr	Pro	Pro	Gln	Gly	Ala	Ser
			435					440					445			
	Gly	Trp	Asn	Thr	Asn	Leu	Met	Arg	Gly	Ser	Val	Ser	Gly	Leu	Ser	Phe
		450					455					460				
	Leu	Gln	Arg	Asp	Gly	Thr	Arg	Leu	Ser	Ala	Gly	Met	Gly	Gly	Gly	Phe
30	465					470					475					480
	Ala	Asp	Thr	Ile	Tyr	Ser	Leu	Pro	Ala	Thr	His	Tyr	Leu	Ser	Tyr	Leu
				485						490					495	
	Tyr	Gly	Thr	Pro	Tyr	Gln	Thr	Ser	Asp	Asn	Tyr	Ser	Gly	His	Val	Gly
			500						505					510		
35	Ala	Leu	Val	Gly	Val	Ser	Thr	Pro	Gln	Glu	Ala	Thr	Leu	Pro	Asn	Ile
			515					520					525			
	Ile	Gly	Gln	Pro	Asp	Glu	Gln	Gly	Asn	Val	Ser	Thr	Met	Gly	Phe	Pro
		530					535					540				
	Phe	Glu	Lys	Ala	Ser	Tyr	Gly	Gly	Thr	Val	Val	Lys	Glu	Trp	Leu	Asn
40	545					550					555					560
	Gly	Ala	Asn	Ala	Met	Lys	Leu	Ser	Pro	Gly	Gln	Ser	Ile	Gly	Ile	Pro
				565						570					575	
	Ile	Thr	Asn	Val	Thr	Ser	Gly	Glu	Tyr	Gln	Ile	Arg	Cys	Arg	Tyr	Ala
			580						585					590		
45	Ser	Asn	Asp	Asn	Thr	Asn	Val	Phe	Phe	Asn	Val	Asp	Thr	Gly	Gly	Ala
			595					600					605			
	Asn	Pro	Ile	Phe	Gln	Gln	Ile	Asn	Phe	Ala	Ser	Thr	Val	Asp	Asn	Asn
		610					615					620				
	Thr	Gly	Val	Gln	Gly	Ala	Asn	Gly	Val	Tyr	Val	Val	Lys	Ser	Ile	Ala
	625					630					635					640
50	Thr	Thr	Asp	Asn	Ser	Phe	Thr	Glu	Ile	Pro	Ala	Lys	Thr	Ile	Asn	Val
				645						650					655	
	His	Leu	Thr	Asn	Gln	Gly	Ser	Ser	Asp	Val	Phe	Leu	Asp	Arg	Ile	Glu
				660					665					670		
	Phe	Ile	Pro	Phe	Ser	Leu	Pro	Leu	Ile	Tyr	His	Gly	Ser	Tyr	Asn	Thr
			675					680					685			
55	Ser	Ser	Gly	Ala	Asp	Asp	Val	Leu	Trp	Ser	Ser	Ser	Asn	Met	Asn	Tyr
		690					695						700			

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Tyr Asp Ile Ile Val Asn Gly Gln Ala Asn Ser Ser Ser Ile Ala Ser
 705 710 715 720
 Ser Met His Leu Leu Asn Lys Gly Lys Val Ile Lys Thr Ile Asp Ile
 725 730 735
 5 Pro Gly His Ser Glu Thr Phe Phe Ala Thr Phe Pro Val Pro Glu Gly
 740 745 750
 Phe Asn Glu Val Arg Ile Leu Ala Gly Leu Pro Glu Val Ser Gly Asn
 755 760 765
 10 Ile Thr Val Gln Ser Asn Asn Pro Pro Gln Pro Ser Asn Asn Gly Gly
 770 775 780
 Gly Asp Gly Gly Gly Asn Gly Gly Gly Asp Gly Gly Gln Tyr Asn Phe
 785 790 795 800
 Ser Leu Ser Gly Ser Asp His Thr Thr Ile Tyr His Gly Lys Leu Glu
 805 810 815
 15 Thr Gly Ile His Val Gln Gly Asn Tyr Thr Tyr Thr Gly Thr Pro Val
 820 825 830
 Leu Ile Leu Asn Ala Tyr Arg Asn Asn Thr Val Val Ser Ser Ile Pro
 835 840 845
 Val Tyr Ser Pro Phe Asp Ile Thr Ile Gln Thr Glu Ala Asp Ser Leu
 850 855 860
 20 Glu Leu Glu Leu Gln Pro Arg Tyr Gly Phe Ala Thr Val Asn Gly Thr
 865 870 875 880
 Ala Thr Val Lys Ser Pro Asn Val Asn Tyr Asp Arg Ser Phe Lys Leu
 885 890 895
 Pro Ile Asp Leu Gln Asn Ile Thr Thr Gln Val Asn Ala Leu Phe Ala
 900 905 910
 25 Ser Gly Thr Gln Asn Met Leu Ala His Asn Val Ser Asp His Asp Ile
 915 920 925
 Glu Glu Val Val Leu Lys Val Asp Ala Leu Ser Asp Glu Val Phe Gly
 930 935 940
 30 Asp Glu Lys Lys Ala Leu Arg Lys Leu Val Asn Gln Ala Lys Arg Leu
 945 950 955 960
 Ser Arg Ala Arg Asn Leu Leu Ile Gly Gly Ser Phe Glu Asn Trp Asp
 965 970 975
 Ala Trp Tyr Lys Gly Arg Asn Val Val Thr Val Ser Asp His Glu Leu
 980 985 990
 35 Phe Lys Ser Asp His Val Leu Leu Pro Pro Pro Gly Leu Ser Pro Ser
 995 1000 1005
 Tyr Ile Phe Gln Lys Val Glu Glu Ser Lys Leu Lys Pro Asn Thr Arg
 1010 1015 1020
 Tyr Ile Val Ser Gly Phe Ile Ala His Gly Lys Asp Leu Glu Ile Val
 1025 1030 1035 1040
 40 Val Ser Arg Tyr Gly Gln Glu Val Gln Lys Val Val Gln Val Pro Tyr
 1045 1050 1055
 Gly Glu Ala Phe Pro Leu Thr Ser Asn Gly Pro Val Cys Cys Pro Pro
 1060 1065 1070
 Arg Ser Thr Ser Asn Gly Thr Leu Gly Asp Pro His Phe Phe Ser Tyr
 1075 1080 1085
 45 Ser Ile Asp Val Gly Ala Leu Asp Leu Gln Ala Asn Pro Gly Ile Glu
 1090 1095 1100
 Phe Gly Leu Arg Ile Val Asn Pro Thr Gly Met Ala Arg Val Ser Asn
 1105 1110 1115 1120
 50 Leu Glu Ile Arg Glu Asp Arg Pro Leu Ala Ala Asn Glu Ile Arg Gln
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 1140 1145 1150
 Ala Glu Val Thr Ser Leu Ile Gln Pro Val Ile Asn Arg Ile Asn Gly
 1155 1160 1165
 55 Leu Tyr Glu Asn Gly Asn Trp Asn Gly Ser Ile Arg Ser Asp Ile Ser
 1170 1175 1180

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Tyr Gln Asn Ile Asp Ala Ile Val Leu Pro Thr Leu Pro Lys Leu Arg
 1185 1190 1195 1200
 His Trp Phe Met Ser Asp Arg Phe Ser Glu Gln Gly Asp Ile Met Ala
 1205 1210 1215
 Lys Phe Gln Gly Ala Leu Asn Arg Ala Tyr Ala Gln Leu Glu Gln Ser
 1220 1225 1230
 Thr Leu Leu His Asn Gly His Phe Thr Lys Asp Ala Ala Asn Trp Thr
 1235 1240 1245
 Ile Glu Gly Asp Ala His Gln Ile Thr Leu Glu Asp Gly Arg Arg Val
 1250 1255 1260
 Leu Arg Leu Pro Asp Trp Ser Ser Ser Val Ser Gln Met Ile Glu Ile
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 Glu Asn Phe Asn Pro Asp Lys Glu Tyr Asn Leu Val Phe His Gly Gln
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 Gly Glu Gly Thr Val Thr Leu Glu His Gly Glu Glu Thr Lys Tyr Ile
 1300 1305 1310
 Glu Thr His Thr His His Phe Ala Asn Phe Thr Thr Ser Gln Arg Gln
 1315 1320 1325
 Gly Leu Thr Phe Glu Ser Asn Lys Val Thr Val Thr Ile Ser Ser Glu
 1330 1335 1340
 Asp Gly Glu Phe Leu Val Asp Asn Ile Ala Leu Val Glu Ala Pro Leu
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<400> 11

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 Pro Ala Asp Leu Thr Ala Tyr Glu Gln Leu Leu Lys Asn Leu Glu Lys
 35 40 45
 Gly Ile Asn Ala Gly Thr Tyr Ser Lys Ala Ile Ala Asp Val Leu Lys
 50 55 60
 Gly Ile Phe Ile Asp Asp Thr Ile Asn Tyr Gln Thr Tyr Val Asn Ile
 65 70 75 80
 Gly Leu Ser Leu Ile Thr Leu Ala Val Pro Glu Ile Gly Ile Phe Thr
 85 90 95
 Pro Phe Ile Gly Leu Phe Phe Ala Ala Leu Asn Lys His Asp Ala Pro
 100 105 110
 Pro Pro Pro Asn Ala Lys Asp Ile Phe Glu Ala Met Lys Pro Ala Ile
 115 120 125
 Gln Glu Met Ile Asp Arg Thr Leu Thr Ala Asp Glu Gln Thr Phe Leu
 130 135 140
 Asn Gly Glu Ile Ser Gly Leu Gln Asn Leu Ala Ala Arg Tyr Gln Ser
 145 150 155 160
 Thr Met Asp Asp Ile Gln Ser His Gly Gly Phe Asn Lys Val Asp Ser
 165 170 175
 Gly Leu Ile Lys Lys Phe Thr Asp Glu Val Leu Ser Leu Asn Ser Phe
 180 185 190
 Tyr Thr Asp Arg Leu Pro Val Phe Ile Thr Asp Asn Thr Ala Asp Arg
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	Thr	Leu	Leu	Gly	Leu	Pro	Tyr	Tyr	Ala	Ile	Leu	Ala	Ser	Met	His	Leu
	210						215					220				
	Met	Leu	Leu	Arg	Asp	Ile	Ile	Thr	Lys	Gly	Pro	Thr	Trp	Asp	Ser	Lys
	225					230					235					240
5	Ile	Asn	Phe	Thr	Pro	Asp	Ala	Ile	Asp	Ser	Phe	Lys	Thr	Asp	Ile	Lys
					245					250					255	
	Asn	Asn	Ile	Lys	Leu	Tyr	Ser	Lys	Thr	Ile	Tyr	Asp	Val	Phe	Gln	Lys
				260					265					270		
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			275					280					285			
	Lys	Gln	Lys	Tyr	Ile	Glu	Ile	Met	Thr	Thr	His	Cys	Leu	Asp	Phe	Ala
	290						295					300				
	Arg	Leu	Phe	Pro	Thr	Phe	Asp	Pro	Asp	Leu	Tyr	Pro	Thr	Gly	Ser	Gly
	305					310					315					320
15	Asp	Ile	Ser	Leu	Gln	Lys	Thr	Arg	Arg	Ile	Leu	Ser	Pro	Phe	Ile	Pro
					325					330					335	
	Ile	Arg	Thr	Ala	Asp	Gly	Leu	Thr	Leu	Asn	Asn	Thr	Ser	Ile	Asp	Thr
				340					345					350		
	Ser	Asn	Trp	Pro	Asn	Tyr	Glu	Asn	Gly	Asn	Gly	Ala	Phe	Pro	Asn	Pro
			355					360					365			
20	Lys	Glu	Arg	Ile	Leu	Lys	Gln	Phe	Lys	Leu	Tyr	Pro	Ser	Trp	Arg	Ala
	370						375					380				
	Ala	Gln	Tyr	Gly	Gly	Leu	Leu	Gln	Pro	Tyr	Leu	Trp	Ala	Ile	Glu	Val
	385					390					395					400
	Gln	Asp	Ser	Val	Glu	Thr	Arg	Leu	Tyr	Gly	Gln	Leu	Pro	Ala	Val	Asp
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25	Pro	Gln	Ala	Gly	Pro	Asn	Tyr	Val	Ser	Ile	Asp	Ser	Ser	Asn	Pro	Ile
				420					425					430		
	Ile	Gln	Ile	Asn	Met	Asp	Thr	Trp	Lys	Thr	Pro	Pro	Gln	Gly	Ala	Ser
			435					440					445			
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	450						455					460				
	Leu	Gln	Arg	Asp	Gly	Thr	Arg	Leu	Ser	Ala	Gly	Met	Gly	Gly	Gly	Phe
	465					470					475					480
	Ala	Asp	Thr	Ile	Tyr	Ser	Leu	Pro	Ala	Thr	His	Tyr	Leu	Ser	Tyr	Leu
					485					490					495	
35	Tyr	Gly	Thr	Pro	Tyr	Gln	Thr	Ser	Asp	Asn	Tyr	Ser	Gly	His	Val	Gly
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			515					520					525			
	Ile	Gly	Gln	Pro	Asp	Glu	Gln	Gly	Asn	Val	Ser	Thr	Met	Gly	Phe	Pro
	530						535					540				
40	Phe	Glu	Lys	Ala	Ser	Tyr	Gly	Gly	Thr	Val	Val	Lys	Glu	Trp	Leu	Asn
	545					550					555					560
	Gly	Ala	Asn	Ala	Met	Lys	Leu	Ser	Pro	Gly	Gln	Ser	Ile	Gly	Ile	Pro
					565					570					575	
45	Ile	Thr	Asn	Val	Thr	Ser	Gly	Glu	Tyr	Gln	Ile	Arg	Cys	Arg	Tyr	Ala
			580					585					590			
	Ser	Asn	Asp	Asn	Thr	Asn	Val	Phe	Phe	Asn	Val	Asp	Thr	Gly	Gly	Ala
			595					600					605			
	Asn	Pro	Ile	Phe	Gln	Gln	Ile	Asn	Phe	Ala	Ser	Thr	Val	Asp	Asn	Asn
			610				615					620				
50	Thr	Gly	Val	Gln	Gly	Ala	Asn	Gly	Val	Tyr	Val	Val	Lys	Ser	Ile	Ala
	625					630					635					640
	Thr	Thr	Asp	Asn	Ser	Phe	Thr	Val	Lys	Ile	Pro	Ala	Lys	Thr	Ile	Asn
					645					650					655	
	Val	His	Leu	Thr	Asn	Gln	Gly	Ser	Ser	Asp	Val	Phe	Leu	Asp	Arg	Ile
				660				665					670			
55	Glu	Phe	Val	Pro	Ile	Leu	Glu	Ser	Asn	Thr	Val	Thr	Ile	Phe	Asn	Asn
			675					680					685			

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	Ser	Tyr	Thr	Thr	Gly	Ser	Ala	Asn	Leu	Ile	Pro	Ala	Ile	Ala	Pro	Leu
	690						695				700					
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	705					710					715					720
5	Gly	Arg	Thr	Thr	Pro	Asn	Ser	Asp	Asp	Ala	Leu	Leu	Arg	Phe	Phe	Lys
					725					730						735
	Thr	Asn	Tyr	Asp	Thr	Gln	Thr	Ile	Pro	Ile	Pro	Gly	Ser	Gly	Lys	Asp
				740					745					750		
	Phe	Thr	Asn	Thr	Leu	Glu	Ile	Gln	Asp	Ile	Val	Ser	Ile	Asp	Ile	Phe
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	Val	Gly	Ser	Gly	Leu	His	Gly	Ser	Asp	Gly	Ser	Ile	Lys	Leu	Asp	Phe
	770						775					780				
	Thr	Asn	Asn	Asn	Ser	Gly	Ser	Gly	Gly	Ser	Pro	Lys	Ser	Phe	Thr	Glu
	785					790					795					800
15	Gln	Asn	Asp	Leu	Glu	Asn	Ile	Thr	Thr	Gln	Val	Asn	Ala	Leu	Phe	Thr
					805					810					815	
	Ser	Asn	Thr	Gln	Asp	Ala	Leu	Ala	Thr	Asp	Val	Ser	Asp	His	Asp	Ile
				820					825					830		
	Glu	Glu	Val	Val	Leu	Lys	Val	Asp	Ala	Leu	Ser	Asp	Glu	Val	Phe	Gly
			835					840					845			
20	Lys	Glu	Lys	Lys	Thr	Leu	Arg	Lys	Phe	Val	Asn	Gln	Ala	Lys	Arg	Leu
	850						855					860				
	Ser	Lys	Ala	Arg	Asn	Leu	Leu	Val	Gly	Gly	Asn	Phe	Asp	Asn	Leu	Asp
	865				870				875							880
	Ala	Trp	Tyr	Arg	Gly	Arg	Asn	Val	Val	Asn	Val	Ser	Asn	His	Glu	Leu
					885					890					895	
25	Leu	Lys	Ser	Asp	His	Val	Leu	Leu	Pro	Pro	Pro	Gly	Leu	Ser	Pro	Ser
				900					905					910		
	Tyr	Ile	Phe	Gln	Lys	Val	Glu	Glu	Ser	Lys	Leu	Lys	Arg	Asn	Thr	Arg
			915				920						925			
30	Tyr	Thr	Val	Ser	Gly	Phe	Ile	Ala	His	Ala	Thr	Asp	Leu	Glu	Ile	Val
	930						935					940				
	Val	Ser	Arg	Tyr	Gly	Gln	Glu	Ile	Lys	Lys	Val	Val	Gln	Val	Pro	Tyr
	945				950					955						960
	Gly	Glu	Ala	Phe	Pro	Leu	Thr	Ser	Ser	Gly	Pro	Val	Cys	Cys	Ile	Pro
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35	His	Ser	Thr	Ser	Asn	Gly	Thr	Leu	Gly	Asn	Pro	His	Phe	Phe	Ser	Tyr
				980					985					990		
	Ser	Ile	Asp	Val	Gly	Ala	Leu	Asp	Val	Asp	Thr	Asn	Pro	Gly	Ile	Glu
			995					1000					1005			
	Phe	Gly	Leu	Arg	Ile	Val	Asn	Pro	Thr	Gly	Met	Ala	Arg	Val	Ser	Asn
	1010						1015					1020				
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	Val	Gln	Arg	Val	Ala	Arg	Asn	Trp	Arg	Thr	Glu	Tyr	Glu	Lys	Glu	Arg
					1045					1050					1055	
45	Ala	Glu	Val	Thr	Ser	Leu	Ile	Gln	Pro	Val	Ile	Asn	Arg	Ile	Asn	Gly
				1060					1065					1070		
	Leu	Tyr	Asp	Asn	Gly	Asn	Trp	Asn	Gly	Ser	Ile	Arg	Ser	Asp	Ile	Ser
			1075					1080					1085			
	Tyr	Gln	Asn	Ile	Asp	Ala	Ile	Val	Leu	Pro	Thr	Leu	Pro	Lys	Leu	Arg
	1090						1095					1100				
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	1105					1110					1115					1120
	Lys	Phe	Gln	Gly	Ala	Leu	Asn	Arg	Ala	Tyr	Ala	Gln	Leu	Glu	Gln	Asn
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	Thr	Leu	Leu	His	Asn	Gly	His	Phe	Thr	Lys	Asp	Ala	Ala	Asn	Trp	Thr
				1140					1145					1150		
55	Val	Glu	Gly	Asp	Ala	His	Gln	Val	Val	Leu	Glu	Asp	Gly	Lys	Arg	Val
				1155				1160					1165			

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5 Leu Arg Leu Pro Asp Trp Ser Ser Ser Val Ser Gln Thr Ile Glu Ile
 1170 1175 1180
 Glu Asn Phe Asp Pro Asp Lys Glu Tyr Gln Leu Val Phe His Gly Gln
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 Gly Glu Gly Thr Val Thr Leu Glu His Gly Glu Glu Thr Lys Tyr Ile
 1205 1210 1215
 Glu Thr His Thr His His Phe Ala Asn Phe Thr Thr Ser Gln Arg Gln
 1220 1225 1230
 Gly Leu Thr Phe Glu Ser Asn Lys Val Thr Val Thr Ile Ser Ser Glu
 1235 1240 1245
 10 Asp Gly Glu Phe Leu Val Asp Asn Ile Ala Leu Val Glu Ala Pro Leu
 1250 1255 1260
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20 <213> Bacillus thuringiensis

<400> 12

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 His Pro Ile Lys Glu Val Asp Asp Pro Tyr Ser Trp Ser Asn Leu Leu
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 Lys Gly Ile Gln Glu Gly Trp Glu Glu Trp Gly Lys Thr Gly Gln Lys
 35 40 45
 30 Lys Leu Phe Glu Asp His Leu Thr Ile Ala Trp Asn Leu Tyr Lys Thr
 50 55 60
 Gly Lys Leu Asp Tyr Phe Ala Leu Thr Lys Ala Ser Ile Ser Leu Ile
 65 70 75 80
 Gly Phe Ile Pro Gly Ala Glu Ala Ala Val Pro Phe Ile Asn Met Phe
 85 90 95
 35 Val Asp Phe Val Trp Pro Lys Leu Phe Gly Ala Asn Thr Glu Gly Lys
 100 105 110
 Asp Gln Gln Leu Phe Asn Ala Ile Met Asp Ala Val Asn Lys Met Val
 115 120 125
 40 Asp Asn Lys Phe Leu Ser Tyr Asn Leu Ser Thr Leu Asn Lys Thr Ile
 130 135 140
 Glu Gly Leu Gln Gly Asn Leu Gly Leu Phe Gln Asn Ala Ile Gln Val
 145 150 155 160
 Ala Ile Cys Gln Gly Ser Thr Pro Glu Arg Val Asn Phe Asp Gln Asn
 165 170 175
 45 Cys Thr Pro Cys Asn Pro Asn Gln Pro Cys Lys Asp Asp Leu Asp Arg
 180 185 190
 Val Ala Ser Arg Phe Asp Thr Ala Asn Ser Gln Phe Thr Gln His Leu
 195 200 205
 Pro Glu Phe Lys Asn Pro Trp Ser Asp Glu Asn Ser Thr Gln Glu Phe
 210 215 220
 50 Lys Arg Thr Ser Val Glu Leu Thr Leu Pro Met Tyr Thr Thr Val Ala
 225 230 235 240
 Thr Leu His Leu Leu Leu Tyr Glu Gly Tyr Ile Glu Phe Met Thr Lys
 245 250 255
 Trp Asn Phe His Asn Glu Gln Tyr Leu Asn Asn Leu Lys Val Glu Leu
 260 265 270
 55 Gln Gln Leu Ile His Ser Tyr Ser Glu Thr Val Arg Thr Ser Phe Leu
 275 280 285

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	305					310					315					320
5	Ala	Thr	Trp	Pro	Thr	Phe	Asp	Thr	His	Asn	Tyr	His	Gln	Gly	Gly	Lys
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	Leu	Asp	Leu	Thr	Arg	Ile	Ile	Leu	Ser	Asp	Thr	Ala	Gly	Pro	Ile	Glu
				340					345					350		
	Glu	Tyr	Thr	Thr	Gly	Asp	Lys	Thr	Ser	Gly	Pro	Glu	His	Ser	Asn	Ile
10			355					360					365			
	Thr	Pro	Asn	Asn	Ile	Leu	Asp	Thr	Pro	Ser	Pro	Thr	Tyr	Gln	His	Ser
		370					375					380				
	Phe	Val	Ser	Val	Asp	Ser	Ile	Val	Tyr	Ser	Arg	Lys	Glu	Leu	Gln	Gln
	385					390					395					400
15	Leu	Asp	Ile	Ala	Thr	Tyr	Ser	Thr	Asn	Asn	Ser	Asn	Asn	Cys	His	Pro
					405					410					415	
	Tyr	Gly	Leu	Arg	Leu	Ser	Tyr	Thr	Asp	Gly	Ser	Arg	Tyr	Asp	Tyr	Gly
				420					425					430		
	Asp	Asn	Gln	Pro	Asp	Phe	Thr	Thr	Ser	Asn	Asn	Asn	Tyr	Cys	His	Asn
		435						440					445			
20	Ser	Tyr	Thr	Ala	Pro	Ile	Thr	Leu	Val	Asn	Ala	Arg	His	Leu	Tyr	Asn
		450					455					460				
	Ala	Lys	Gly	Ser	Leu	Gln	Asn	Val	Glu	Ser	Leu	Val	Val	Ser	Thr	Val
	465					470					475					480
	Asn	Gly	Gly	Ser	Gly	Ser	Cys	Ile	Cys	Asp	Ala	Trp	Ile	Asn	Tyr	Leu
					485					490					495	
25	Arg	Pro	Pro	Gln	Thr	Ser	Lys	Asn	Glu	Ser	Arg	Pro	Asp	Gln	Lys	Ile
				500					505					510		
	Asn	Val	Leu	Tyr	Pro	Ile	Thr	Glu	Thr	Val	Asn	Lys	Gly	Thr	Gly	Gly
		515						520					525			
30	Asn	Leu	Gly	Val	Ile	Ser	Ala	Tyr	Val	Pro	Met	Glu	Leu	Val	Pro	Glu
		530					535					540				
	Asn	Val	Ile	Gly	Asp	Val	Asn	Ala	Asp	Thr	Lys	Leu	Pro	Leu	Thr	Gln
	545					550					555					560
	Leu	Lys	Gly	Phe	Pro	Phe	Glu	Lys	Tyr	Gly	Ser	Glu	Tyr	Asn	Asn	Arg
				565						570					575	
35	Gly	Ile	Ser	Leu	Val	Arg	Glu	Trp	Ile	Asn	Gly	Asn	Asn	Ala	Val	Lys
				580					585					590		
	Leu	Ser	Asn	Ser	Gln	Ser	Val	Gly	Ile	Gln	Ile	Thr	Asn	Gln	Thr	Lys
		595						600					605			
	Gln	Lys	Tyr	Glu	Ile	Arg	Cys	Arg	Tyr	Ala	Ser	Lys	Gly	Asp	Asn	Asn
		610					615					620				
40	Val	Tyr	Phe	Asn	Val	Asp	Leu	Ser	Glu	Asn	Pro	Phe	Arg	Asn	Ser	Ile
	625					630					635					640
	Ser	Phe	Gly	Ser	Thr	Glu	Ser	Ser	Val	Val	Gly	Val	Gln	Gly	Glu	Asn
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45	Gly	Lys	Tyr	Ile	Leu	Lys	Ser	Ile	Thr	Thr	Val	Glu	Ile	Pro	Ala	Gly
				660					665					670		
	Ser	Phe	Tyr	Val	His	Ile	Thr	Asn	Gln	Gly	Ser	Ser	Asp	Leu	Phe	Leu
		675						680					685			
	Asp	Arg	Ile	Glu	Phe	Val	Pro	Lys	Ile	Gln	Phe	Gln	Phe	Cys	Asp	Asn
		690					695					700				
50	Asn	Asn	Leu	His	Cys	Asp	Cys	Asn	Asn	Pro	Val	Asp	Thr	Asp	Cys	Thr
	705					710					715					720
	Phe	Cys	Cys	Val	Cys	Thr	Ser	Leu	Thr	Asp	Cys	Asp	Cys	Asn	Asn	Pro
				725						730					735	
	Arg	Gly	Leu	Asp	Cys	Thr	Leu	Cys	Cys	Gln	Val	Glu	Asn	Gln	Leu	Pro
				740					745					750		
55	Ser	Phe	Val	Thr	Leu	Thr	Asp	Leu	Gln	Asn	Ile	Thr	Thr	Gln	Val	Asn
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	Ala	Leu	Val	Ala	Ser	Ser	Glu	His	Asp	Thr	Leu	Ala	Thr	Asp	Val	Ser
	770						775					780				
	Asp	Tyr	Glu	Ile	Glu	Glu	Val	Val	Leu	Lys	Val	Asp	Ala	Leu	Ser	Gly
	785				790						795					800
5	Glu	Val	Phe	Gly	Lys	Glu	Lys	Lys	Ala	Leu	Arg	Lys	Leu	Val	Asn	His
				805						810					815	
	Thr	Lys	Arg	Leu	Ser	Lys	Ala	Arg	Asn	Leu	Leu	Ile	Gly	Gly	Asn	Phe
				820					825					830		
	Asp	Asn	Leu	Asp	Ala	Trp	Tyr	Arg	Gly	Arg	Asn	Val	Val	Asn	Val	Ser
			835					840					845			
10	Asp	His	Glu	Leu	Phe	Lys	Ser	Asp	His	Val	Leu	Leu	Pro	Pro	Pro	Thr
	850						855					860				
	Leu	Tyr	Ser	Ser	Tyr	Met	Phe	Gln	Lys	Val	Glu	Glu	Ser	Lys	Leu	Lys
	865					870					875					880
15	Ala	Asn	Thr	Arg	Tyr	Thr	Val	Ser	Gly	Phe	Ile	Ala	His	Ala	Glu	Asp
				885						890					895	
	Leu	Glu	Ile	Val	Val	Ser	Arg	Tyr	Gly	Gln	Glu	Val	Lys	Lys	Val	Val
				900					905					910		
	Gln	Val	Pro	Tyr	Gly	Glu	Ala	Phe	Pro	Leu	Thr	Ser	Arg	Gly	Ala	Ile
			915					920					925			
20	Cys	Cys	Pro	Pro	Arg	Ser	Thr	Ser	Asn	Gly	Lys	Pro	Ala	Asp	Pro	His
	930						935					940				
	Phe	Phe	Ser	Tyr	Ser	Ile	Asp	Val	Gly	Thr	Leu	Asp	Val	Glu	Ala	Asn
	945					950					955					960
	Pro	Gly	Ile	Glu	Leu	Gly	Leu	Arg	Ile	Val	Glu	Arg	Thr	Gly	Met	Ala
				965						970					975	
25	Arg	Val	Ser	Asn	Leu	Glu	Ile	Arg	Glu	Asp	Arg	Pro	Leu	Lys	Lys	Asn
				980					985					990		
	Glu	Leu	Arg	Asn	Val	Gln	Arg	Ala	Ala	Arg	Asn	Trp	Arg	Thr	Ala	Tyr
			995					1000					1005			
	Asp	Gln	Glu	Arg	Ala	Glu	Val	Thr	Ala	Leu	Ile	Gln	Pro	Val	Leu	Asn
		1010					1015					1020				
	Gln	Ile	Asn	Ala	Leu	Tyr	Glu	Asn	Glu	Asp	Trp	Asn	Gly	Ala	Ile	Arg
	1025					1030					1035					1040
	Ser	Gly	Val	Ser	Tyr	His	Asp	Leu	Glu	Ala	Ile	Val	Leu	Pro	Thr	Leu
				1045						1050					1055	
35	Pro	Lys	Leu	Asn	His	Trp	Phe	Met	Ser	Asp	Met	Leu	Gly	Glu	Gln	Gly
				1060					1065					1070		
	Ser	Ile	Leu	Ala	Gln	Phe	Gln	Glu	Ala	Leu	Asp	Arg	Ala	Tyr	Thr	Gln
			1075					1080					1085			
	Leu	Glu	Glu	Ser	Thr	Ile	Leu	His	Asn	Gly	His	Phe	Thr	Thr	Asp	Ala
		1090					1095					1100				
40	Ala	Asn	Trp	Thr	Ile	Glu	Gly	Asp	Ala	His	His	Ala	Ile	Leu	Glu	Asp
	1105					1110					1115					1120
	Gly	Arg	Arg	Val	Leu	Arg	Leu	Pro	Asp	Trp	Ser	Ser	Ser	Val	Ser	Gln
				1125						1130					1135	
45	Thr	Ile	Glu	Ile	Glu	Asn	Phe	Asp	Pro	Asp	Lys	Glu	Tyr	Gln	Leu	Val
				1140					1145					1150		
	Phe	His	Ala	Gln	Gly	Glu	Gly	Thr	Val	Ser	Leu	Gln	His	Gly	Glu	Glu
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[illegible]

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	225				230				235				240			
	Ile	Leu	His	Gln	Gly	Tyr	Ile	Gln	Phe	Val	Glu	Arg	Trp	Lys	Ser	Val
					245					250					255	
5	Asp	Tyr	Asp	Glu	Ala	Phe	Ile	Asn	Gln	Thr	Lys	Ala	Asp	Leu	Gln	His
				260					265					270		
	Arg	Ile	Gln	Glu	Tyr	Ser	Thr	Thr	Val	Ser	Thr	Thr	Phe	Glu	Lys	Phe
			275					280					285			
	Lys	Pro	Thr	Leu	Ser	Asn	Lys	Lys	Ser	Ser	Ile	Asn	Thr	Tyr	Asn	Lys
		290					295					300				
10	Tyr	Val	Arg	Ser	Met	Thr	Leu	Asn	Cys	Leu	Asp	Ile	Ala	Ala	Thr	Trp
	305					310					315				320	
	Pro	Thr	Leu	Asp	Asn	Val	Asn	Tyr	Pro	Ser	Asn	Val	Glu	Ile	Gln	Leu
					325					330					335	
	Asp	Gln	Thr	Arg	Leu	Val	Phe	Ser	Asn	Leu	Val	Gly	Pro	Phe	Glu	Gly
				340					345					350		
15	Asn	Asp	Asp	Ile	Ser	Thr	Tyr	Thr	Arg	Arg	Ser	Ile	Met	Asn	Tyr	Ser
			355					360					365			
	Lys	Gly	Asp	Thr	Pro	Gly	Asp	Val	Asn	Ser	Ala	Ile	Gln	Ser	Leu	Arg
		370					375					380				
	Tyr	Pro	Arg	Leu	Glu	Leu	Ser	Lys	Val	Gln	Phe	Tyr	Thr	His	Asp	Gln
20	385					390					395				400	
	Arg	Ser	Asn	Gly	Val	Arg	His	Cys	Tyr	Thr	Ser	Gly	Phe	Asn	Leu	Thr
					405					410					415	
	Phe	Asn	Asp	Asn	Ser	Ser	Met	Ser	Ala	Lys	Gln	Asp	Glu	Ser	Ala	Thr
				420					425					430		
25	Ala	Asp	Ser	Pro	Pro	Leu	Thr	Ala	Pro	Ile	Lys	Asn	Met	Asn	Ala	Asn
			435					440					445			
	Ser	Gln	Asn	Ser	Gln	Tyr	Tyr	Asp	Tyr	Ser	Ser	Ile	Asn	Ile	Asp	Asn
		450					455					460				
	Gln	Gly	Gly	Gly	Gly	Cys	Ser	Ala	Phe	Pro	Ser	Tyr	Gln	Ser	Asn	Asn
30	465					470					475				480	
	Pro	Ile	Leu	Pro	Asn	Gln	Lys	Ile	Asn	Val	Phe	Tyr	Pro	Tyr	Gly	Ser
					485					490					495	
	Ser	Ala	His	Pro	Ile	Asp	Pro	His	Thr	Thr	Asp	Pro	Asp	Thr	Trp	Phe
				500				505					510			
	Lys	Leu	Gly	Tyr	Val	Ser	Ser	His	Ile	Pro	Tyr	Asp	Leu	Thr	Pro	Gln
35			515					520					525			
	Asn	Val	Ile	Gly	Glu	Ile	Asp	Gln	Asp	Thr	Lys	Gln	Pro	Ser	Leu	Ile
		530					535					540				
	Leu	Lys	Gly	Phe	Pro	Ala	Glu	Lys	Gly	Tyr	Gly	Gly	Ser	Ile	Glu	Tyr
	545					550					555				560	
40	Val	Ser	Glu	Pro	Leu	Asn	Gly	Ala	Asn	Ala	Ala	Lys	Leu	Thr	Leu	Asn
					565					570					575	
	Gln	Ile	Leu	Tyr	Met	Gln	Val	Thr	Asn	Leu	Thr	Thr	Gln	Lys	Tyr	Gln
				580					585					590		
	Ile	Arg	Leu	Arg	Tyr	Ala	Thr	Lys	Asn	Asp	Thr	Thr	Ala	Ser	Val	Trp
				595				600					605			
45	Phe	His	Ile	Ile	Gly	Pro	Asn	Asn	Gln	Asp	Ile	Ile	Asn	His	Ser	Pro
		610					615					620				
	Asp	Ile	Pro	Pro	Arg	Ser	Asn	Asn	Lys	Met	Phe	Val	Gln	Gly	Glu	Asn
	625					630					635				640	
	Gly	Lys	Tyr	Val	Leu	Asp	Thr	Leu	Val	Asp	Ser	Ile	Glu	Leu	Pro	Ser
50					645					650					655	
	Gly	Gln	Leu	Thr	Ile	Leu	Ile	Gln	Asn	Ile	Asn	Pro	Asp	Gln	Asp	Leu
				660					665					670		
	Phe	Leu	Asp	Arg	Ile	Glu	Phe	Val	Pro	Ile	Pro	Thr	Leu	Pro	Thr	Asn
			675					680					685			
55	Pro	Asn	Ile	Ser	Ile	Pro	Lys	Thr	Asp	Thr	Ser	Pro	Lys	Asp	Ser	Lys
		690					695					700				
	Val	Leu	Trp	Glu	Ala	Ser	Pro	Asp	Ile	Pro	Ile	Ala	Asn	Thr	Ile	Thr

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	705		710		715		720
	Leu Thr Gly Ser Val Tyr Asp Phe Ala Asp Ile Thr Phe Glu Leu Tyr						
		725		730			735
5	Lys Asn Gly Asn Met Val Thr Ser Tyr Pro Ile Lys Gly Pro Gly Pro						
		740		745			750
	Ile Pro His Arg Ser His Gly Asn Tyr Val Ser Cys Ser Gln Gly Ile						
		755		760			765
	Leu Ser Tyr Asn Tyr Glu Asn Lys Pro Val Leu Asp Gly Phe Asp Gln						
		770		775			780
10	Leu Arg Ile Asn Ile Asn Ser Asp Pro Ser Phe Tyr Asp Ser Asn Ser						
		785		790			795
	Gly Cys Asp Thr Lys Asn Gln Tyr Ser Ala Glu Ile Lys Ile Asn Pro						
			805		810		815
	Asn Leu Ser Ala Thr Thr Asp Leu Glu Lys Ile Thr Asn Gln Val Asn						
		820		825			830
15	Gln Leu Phe Thr Ser Ser Ser Gln Thr Glu Leu Ala Asn Thr Ile Thr						
		835		840			845
	Asp Tyr Arg Ile Asp Gln Ile Val Met Lys Val Asp Ala Leu Ser Asn						
		850		855			860
	Asn Val Phe Gly Val Glu Lys Lys Ala Leu Arg Lys Leu Val Asn Gln						
20		865		870			875
	Ala Lys Gln Leu Ser Lys Ala Arg Asn Val Leu Ala Gly Gly Asn Phe						
			885		890		895
	Glu Lys Gly His Glu Trp Val Leu Gly Arg Glu Ala Thr Met Ile Ala						
		900		905			910
25	Asn His Glu Leu Phe Lys Gly Asp His Leu Leu Leu Pro Pro Pro Thr						
		915		920			925
	Leu Tyr Pro Ser Tyr Ala Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys						
		930		935			940
	Ser Asn Thr Arg Tyr Thr Val Ser Gly Phe Ile Ala Gln Ser Glu His						
30		945		950			955
	Leu Glu Val Ile Val Ser Arg Tyr Gly Lys Glu Val His Asp Met Leu						
			965		970		975
	Asp Val Pro Tyr Glu Glu Ala Leu Pro Ile Ser Ser Asp Glu Ser Pro						
		980		985			990
35	Asn Cys Cys Lys Pro Ala Thr Cys Gln Cys Pro Ser Cys Asp Gly Ser						
		995		1000			1005
	Gln Pro Asp Ser His Phe Phe Ser Tyr Ser Ile Asp Val Gly Ser Val						
		1010		1015			1020
	Gln Ser Asp Val Asn Leu Gly Ile Glu Phe Gly Leu Arg Ile Ala Lys						
		1025		1030			1035
40	Pro Asn Gly Phe Ala Lys Ile Ser Asn Leu Glu Ile Lys Glu Asp Arg						
			1045		1050		1055
	Pro Leu Thr Asp Gln Glu Ile Lys Lys Ile Gln Arg Lys Glu Gln Lys						
		1060		1065			1070
	Trp Lys Lys Ala Phe Asp Gln Glu Gln Ala Glu Val Ala Ala Thr Phe						
		1075		1080			1085
45	Gln Pro Thr Leu Asp Gln Ile Asn Ala Leu Tyr Gln Asn Glu Asp Trp						
		1090		1095			1100
	Asn Gly Ser Leu His Pro His Val Thr Tyr Gln His Leu Ser Ala Val						
		1105		1110			1115
	Val Leu Pro Thr Leu Pro Lys Gln Arg His Trp Phe Met Glu Asp Arg						
50		1125		1130			1135
	Glu Gly Glu His Tyr Gly Val Thr Gln Gln Phe Gln Gln Ala Leu Asp						
		1140		1145			1150
	Arg Gly Phe Gln Gln Ile Glu Glu Gln Asn Leu Ile His Asn Gly Ser						
		1155		1160			1165
55	Phe Ala Asn Gly Leu Thr Asp Trp Thr Val Thr Gly Asp Ala Gln Leu						
		1170		1175			1180
	Thr Ile Phe Asp Glu Asp Pro Val Leu Glu Leu Ala His Trp Asp Ala						

	1185		1190		1195		1200									
	Ser	Val	Ser	Gln	Thr	Ile	Glu	Ile	Met	Asp	Phe	Glu	Glu	Glu	Thr	Glu
				1205				1210							1215	
5	Tyr	Lys	Leu	Arg	Val	Arg	Gly	Lys	Gly	Lys	Gly	Thr	Val	Thr	Val	Gln
			1220					1225						1230		
	His	Gly	Glu	Glu	Glu	Leu	Glu	Thr	Met	Thr	Phe	Asn	Thr	Thr	Ser	Phe
		1235						1240					1245			
	Thr	Thr	Gln	Glu	Gln	Thr	Phe	Tyr	Phe	Glu	Gly	Asp	Thr	Val	Asp	Val
		1250					1255					1260				
10	His	Val	Gln	Ser	Glu	Asn	Asn	Thr	Phe	Leu	Val	Asp	Ser	Val	Glu	Leu
	1265					1270					1275				1280	
	Ile	Glu	Val	Val	Glu	Glu										
				1285												

Claims

1. A nucleic acid molecule selected from the group consisting of:

- a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, or 5, or a complement thereof;
- b) a nucleic acid molecule comprising a nucleotide sequence having at least 80% sequence identity to the nucleotide sequence of SEQ ID NO: 1, 3, or 5, or a complement thereof, wherein said nucleotide sequence encodes a polypeptide having pesticidal activity;
- c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, 4, or 6;
- d) a nucleic acid molecule which encodes a polypeptide comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 2, 4, or 6, wherein said nucleotide sequence encodes a polypeptide having pesticidal activity; and,
- e) the delta endotoxin nucleotide sequence of the DNA insert of the plasmid deposited as Accession No. NRRL B-30805, NRRL B-30809, NRRL B-30808, or a complement thereof;

optionally wherein said nucleotide sequence is a synthetic sequence that has been designed for expression in a plant.

2. The nucleic acid molecule of claim 1, wherein said nucleotide sequence is operably linked to a promoter capable of directing expression of the nucleotide sequence in a plant cell.

3. A vector comprising the nucleic acid molecule of claim 1 or 2, optionally further comprising a nucleic acid molecule encoding a heterologous polypeptide.

4. A plant or a bacterial host cell comprising the nucleic acid molecule of claim 2 or the vector of claim 3.

5. An isolated polypeptide with pesticidal activity, selected from the group consisting of:

- a) a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, 4, or 6;
- b) a polypeptide comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 2, 4, or 6, wherein said polypeptide has pesticidal activity;
- c) a polypeptide that is encoded by the nucleotide sequence of SEQ ID NO: 1, 3, or 5;
- d) a polypeptide that is encoded by a nucleotide sequence that is at least 80% identical to the nucleotide sequence of SEQ ID NO: 1, 3, or 5, wherein said polypeptide has pesticidal activity; and,
- e) a polypeptide encoded by the delta endotoxin nucleotide sequence of the DNA insert of the plasmid deposited as Accession No. NRRL B-30805, NRRL B-30809, or NRRL B-30808.

6. The polypeptide of claim 5 further comprising heterologous amino acid sequences.

7. A composition comprising the polypeptide of claim 5, optionally wherein said composition comprises a powder, dust, pellet, granule, spray, emulsion, colloid, and solution and wherein said composition is prepared by desiccation, lyophilization, homogenization, extraction, filtration, centrifugation, sedimentation, or concentration of a culture of the bacterial host cell of claim 4.

8. The composition of claim 7, comprising from about 1% to about 99% by weight of said polypeptide.
9. A method for killing or controlling a lepidopteran, coleopteran, or nematode pest population comprising contacting said population with, or feeding to said pest, a pesticidally-effective amount of a polypeptide of claim 5.

10. A method for producing a polypeptide with pesticidal activity, comprising culturing the bacterial host cell of claim 4 under conditions in which a nucleic acid molecule encoding the polypeptide is expressed, said polypeptide being selected from the group consisting of:

- a) a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, 4, or 6;
- b) a polypeptide encoded by the nucleic acid sequence of SEQ ID NO: 1, 3, or 5;
- c) a polypeptide comprising an amino acid sequence having at least 80% sequence identity to a polypeptide with the amino acid sequence of SEQ ID NO: 2, 4, or 6, wherein said polypeptide has pesticidal activity;
- d) a polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence having at least 80% sequence identity to the nucleic acid sequence of SEQ ID NO: 1, 3, or 5, wherein said polypeptide has pesticidal activity; and,
- e) a polypeptide encoded by the delta endotoxin nucleotide sequence of the DNA insert of the plasmid deposited as Accession No. NRRL B-30805, NRRL B-30809, or NRRL B-30808.

11. A plant having stably incorporated into its genome a DNA construct comprising a nucleotide sequence that encodes a protein having pesticidal activity, wherein said nucleotide sequence is selected from the group consisting of:

- a) the nucleotide sequence of SEQ ID NO: 1, 3, or 5;
- b) a nucleotide sequence having at least 80% sequence identity to the nucleotide sequence of SEQ ID NO: 1, 3, or 5, wherein said nucleotide sequence encodes a polypeptide having pesticidal activity;
- c) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, 4, or 6;
- d) a nucleotide sequence encoding a polypeptide having at least 80% amino acid sequence identity to the amino acid sequence of SEQ ID NO: 2, 4, or 6, wherein said nucleotide sequence encodes a polypeptide having pesticidal activity; and,
- e) the delta endotoxin nucleotide sequence of the DNA insert of the plasmid deposited as Accession No. NRRL B-30805, NRRL B-30809, or NRRL B-30808, or a complement thereof;

wherein said nucleotide sequence is operably linked to a promoter that drives expression of a coding sequence in a plant cell.

12. The plant of claim 11, wherein said plant is selected from the group consisting of maize, sorghum, wheat, cabbage, sunflower, tomato, crucifers, peppers, potato, cotton, rice, soybean, sugarbeet, sugarcane, tobacco, barley, and oilseed rape.

13. A Transformed seed of the plant of claim 12, wherein said seed comprises incorporated in the genome a construct as defined in claim 11.

14. A method for protecting a plant from a pest, comprising introducing into said plant or cell thereof at least one expression vector comprising a nucleotide sequence that encodes a pesticidal polypeptide, wherein said nucleotide sequence is selected from the group consisting of:

- a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, or 5;
- b) a nucleic acid molecule comprising a nucleotide sequence having at least 80% sequence identity to the nucleotide sequence of SEQ ID NO: 1, 3, or 5, wherein said nucleotide sequence encodes a polypeptide having pesticidal activity;
- c) a nucleic acid molecule that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, 4, or 6;
- d) a nucleic acid molecule that encodes a polypeptide having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 2, 4, or 6, wherein said nucleotide sequence encodes a polypeptide having pesticidal activity; and,
- e) the delta endotoxin nucleotide sequence of the DNA insert of the plasmid deposited as Accession No. NRRL B-30805, NRRL B-30809, NRRL B-30808, or a complement thereof.

15. The method of claim 14, wherein said plant produces a pesticidal polypeptide having pesticidal activity against a lepidopteran, coleopteran, or nematode pest.

Patentansprüche

1. Nukleinsäuremolekül, ausgewählt aus der Gruppe, bestehend aus:

- a) einem Nukleinsäuremolekül, das die Nukleotidsequenz von SEQ ID NO:1, 3, oder 5 oder ein Komplement davon umfasst;
- b) einem Nukleinsäuremolekül, umfassend eine Nukleotidsequenz mit wenigstens 80% Sequenzidentität zu der Nukleotidsequenz von SEQ ID NO:1, 3 oder 5 oder einem Komplement davon, wobei die Nukleotidsequenz ein Polypeptid mit Pestizidaktivität codiert;
- c) einem Nukleinsäuremolekül, das ein Polypeptid codiert, das die Aminosäuresequenz von SEQ ID NO:2,4 oder 6 umfasst;
- d) einem Nukleinsäuremolekül, das ein Polypeptid codiert, das eine Aminosäuresequenz umfasst, die wenigstens 80% Sequenzidentität zu der Aminosäuresequenz von SEQ ID NO:2, 4 oder 6 hat, wobei die Nukleotidsequenz ein Polypeptid mit Pestizidaktivität codiert, und
- e) der Delta-Endotoxin-Nukleotidsequenz des DNA-Inserts des Plasmids, hinterlegt als Zugangsnummer NRRL B-30805, NRRL B-30809, NRRL B-30808, oder einem Komplement davon;

wobei die Nukleotidsequenz gegebenenfalls eine synthetische Sequenz ist, die zur Expression in einer Pflanze entwickelt wurde.

2. Nukleinsäuremolekül gemäß Anspruch 1, wobei die Nukleotidsequenz funktionsfähig mit einem Promotor verknüpft ist, der fähig ist, eine Expression der Nukleotidsequenz in einer Pflanzenzelle zu steuern.

3. Vektor, umfassend das Nukleinsäuremolekül gemäß Anspruch 1 oder 2, gegebenenfalls außerdem umfassend ein Nukleinsäuremolekül, das ein heterologes Polypeptid codiert.

4. Pflanze oder bakterielle Wirtszelle, die das Nukleinsäuremolekül gemäß Anspruch 2 oder den Vektor gemäß Anspruch 3 umfasst.

5. Isoliertes Polypeptid mit Pestizidaktivität, ausgewählt aus der Gruppe, bestehend aus:

- a) einem Polypeptid, umfassend die Aminosäuresequenz von SEQ ID NO:2, 4 oder 6;
- b) einem Polypeptid, umfassend eine Aminosäuresequenz, die wenigstens 80% Sequenzidentität zu der Aminosäuresequenz von SEQ ID NO: 2, 4 oder 6 hat, wobei das Polypeptid Pestizidaktivität hat;
- c) einem Polypeptid, das durch die Nukleotidsequenz von SEQ ID NO:1, 3, oder 5 codiert wird;
- d) einem Polypeptid, das durch eine Nukleotidsequenz codiert wird, die wenigstens 80% identisch zu der Nukleotidsequenz von SEQ ID NO:1, 3 oder 5 ist, wobei das Polypeptid Pestizidaktivität hat, und
- e) einem Polypeptid, das durch die Delta-Endotoxin-Nukleotidsequenz des DNA-Inserts des Plasmids, hinterlegt als Zugangsnummer NRRL B-30805, NRRL B-30809 oder NRRL B-30808, codiert wird.

6. Polypeptid gemäß Anspruch 5, das außerdem heterologe Aminosäuresequenzen umfasst.

7. Zusammensetzung, umfassend das Polypeptid gemäß Anspruch 5, wobei gegebenenfalls die Zusammensetzung ein Pulver, ein Stäubemittel, ein Pellet, ein Granulat, ein Spray, eine Emulsion, ein Colloid und eine Lösung umfasst und wobei die Zusammensetzung durch Trocknung, Lyophilisierung, Homogenisierung, Extraktion, Filtration, Zentrifugation, Sedimentation oder Konzentrierung einer Kultur der bakteriellen Wirtszelle gemäß Anspruch 4 hergestellt wurde.

8. Zusammensetzung gemäß Anspruch 7, die etwa 1 Gewichts-% bis etwa 99 Gewichts-% des Polypeptids umfasst.

9. Verfahren zum Abtöten oder Bekämpfen einer Lepidopteren-, Coleopteren- oder Nematoden-Schädlingpopulation, das Inkontaktbringen der Population mit einer pestizidwirksamen Menge eines Polypeptids gemäß Anspruch 5 oder Füttern einer pestizidwirksamen Menge eines Polypeptids gemäß Anspruch 5 an den Schädling umfasst.

10. Verfahren zu Herstellung eines Polypeptids mit Pestizidaktivität, umfassend Kultivieren der Bakterienwirtszelle gemäß Anspruch 4 unter Bedingungen, unter denen ein Nukleinsäuremolekül, das das Polypeptid codiert, exprimiert wird, wobei das Polypeptid ausgewählt ist aus der Gruppe, bestehend aus:

- a) einem Polypeptid, das die Aminosäuresequenz von SEQ ID NO:2, 4 oder 6 umfasst;
- b) einem Polypeptid, das durch die Nukleinsäuresequenz von SEQ ID NO:1, 3 oder 5 codiert wird;
- c) einem Polypeptid, das eine Aminosäuresequenz umfasst, die wenigstens 80% Sequenzidentität zu einem Polypeptid mit der Aminosäuresequenz von SEQ ID NO:2, 4 oder 6 hat, wobei das Polypeptid Pestizidaktivität hat;
- d) einem Polypeptid, das durch ein Nukleinsäuremolekül codiert wird, das eine Nukleotidsequenz umfasst, die wenigstens 80% Sequenzidentität zu der Nukleinsäuresequenz von SEQ ID NO:1, 3 oder 5 hat, wobei das Polypeptid Pestizidaktivität hat, und
- e) einem Polypeptid, das durch die Delta-Endotoxin-Nukleotidsequenz des DNA-Inserts des Plasmids, hinterlegt als Zugangsnummer NRRL B-30805, NRRL B-30809 oder NRRL B-30808, codiert wird.

11. Pflanze, die stabil in ihr Genom ein DNA-Konstrukt eingebaut hat, das eine Nukleotidsequenz umfasst, die ein Protein mit Pestizidaktivität codiert, wobei die Nukleotidsequenz ausgewählt ist aus der Gruppe, bestehend aus:

- a) der Nukleotidsequenz von SEQ ID NO:1, 3 oder 5;
- b) einer Nukleotidsequenz, die wenigstens 80% Sequenzidentität zu der Nukleotidsequenz von SEQ ID NO:1, 3 oder 5 hat, wobei die Nukleotidsequenz ein Polypeptid codiert, das Pestizidaktivität hat;
- c) einer Nukleotidsequenz, die ein Polypeptid codiert, das die Aminosäuresequenz von SEQ ID NO:2, 4 oder 6 umfasst;
- d) einer Nukleotidsequenz, die ein Polypeptid codiert, das wenigstens 80% Aminosäuresequenzidentität zu der Aminosäuresequenz von SEQ ID NO:2, 4 oder 6 hat, wobei die Nukleotidsequenz ein Polypeptid codiert, das Pestizidaktivität hat, und
- e) der Delta-Endotoxin-Nukleotidsequenz des DNA-Inserts des Plasmids, hinterlegt als Zugangsnummer NRRL B-30805, NRRL B-30809 oder NRRL B-30808, oder einem Komplement davon;

wobei die Nukleotidsequenz funktionsfähig mit einem Promotor verknüpft ist, der eine Expression einer codierenden Sequenz in einer Pflanzenzelle steuert.

12. Pflanze gemäß Anspruch 11, wobei die Pflanze aus der Gruppe, bestehend aus Mais, Hirse, Weizen, Kohl, Sonnenblume, Tomate, Cruciferen, Pfeffer bzw. Paprika, Baumwolle, Reis, Sojabohne, Zuckerrübe, Zuckerrohr, Tabak, Gerste und Ölsamenraps, ausgewählt ist.

13. Transformierter Samen der Pflanze gemäß Anspruch 12, wobei der Samen in das Genom ein Konstrukt, wie es in Anspruch 11 definiert ist, eingebaut umfasst.

14. Verfahren zum Schützen einer Pflanze vor einem Schädling, umfassend Einführen wenigstens eines Expressionsvektors, umfassend eine Nukleotidsequenz, die für ein pestizides Polypeptid codiert, in die Pflanze oder eine Zelle davon, wobei die Nukleotidsequenz ausgewählt ist aus der Gruppe, bestehend aus:

- a) einem Nukleinsäuremolekül, das die Nukleotidsequenz von SEQ ID NO:1, 3 oder 5 umfasst;
- b) einem Nukleinsäuremolekül, umfassend eine Nukleotidsequenz, die wenigstens 80% Sequenzidentität zu der Nukleotidsequenz von SEQ ID NO:1, 3 oder 5 hat, wobei die Nukleotidsequenz ein Polypeptid codiert, das Pestizidaktivität hat;
- c) einem Nukleinsäuremolekül, das ein Polypeptid codiert, das die Aminosäuresequenz von SEQ ID NO:2, 4 oder 6 umfasst;
- d) einem Nukleinsäuremolekül, das ein Polypeptid codiert, das wenigstens 80% Sequenzidentität zu der Aminosäuresequenz von SEQ ID NO:2, 4 oder 6 hat, wobei die Nukleotidsequenz ein Polypeptid codiert, das Pestizidaktivität hat, und
- e) der Delta-Endotoxin-Nukleotidsequenz des DNA-Inserts des Plasmids, hinterlegt als Zugangsnummer NRRL B-30805, NRRL B-30809, NRRL B-30808 oder einem Komplement davon.

15. Verfahren gemäß Anspruch 14, wobei die Pflanze ein pestizides Polypeptid produziert, das Pestizidaktivität gegen einen Lepidopteren-, Coleopteren- oder Nematodenschädling hat.

Revendications

1. Molécule d'acide nucléique choisie dans le groupe comprenant:

- a) une molécule d'acide nucléique comprenant la séquence de nucléotides de Séquence n° 1, 3 ou 5, ou une séquence complémentaire de celle-ci;
- b) une molécule d'acide nucléique comprenant une séquence de nucléotides présentant au moins 80% d'identité de séquence avec la séquence de nucléotides de Séquence n° 1, 3 ou 5, ou une séquence complémentaire de celle-ci, ladite séquence de nucléotides codant un polypeptide possédant une activité antiparasitaire;
- c) une molécule d'acide nucléique qui code un polypeptide comprenant la séquence d'acides aminés de Séquence n° 2, 4 ou 6;
- d) une molécule d'acide nucléique qui code un polypeptide comprenant une séquence d'acides aminés présentant au moins 80% d'identité de séquence avec la séquence d'acides aminés de Séquence n° 2, 4 ou 6, ladite séquence de nucléotides codant un polypeptide possédant une activité antiparasitaire; et
- e) la séquence de nucléotides de l'endotoxine delta de l'insert d'ADN du plasmide déposé sous le Numéro d'enregistrement NRRL B-30805, NRRL B-30809, NRRL B-30808 ou une séquence complémentaire de celle-ci;

ladite séquence de nucléotides étant facultativement une séquence synthétique qui a été conçue pour s'exprimer chez une plante.

2. Molécule d'acide nucléique de la revendication 1, ladite séquence de nucléotides étant liée opérationnellement à un promoteur capable de diriger l'expression de la séquence de nucléotides dans une cellule végétale.

3. Vecteur comprenant la molécule d'acide nucléique de la revendication 1 ou 2, comprenant facultativement en outre une molécule d'acide nucléique codant un polypeptide hétérologue.

4. Cellule hôte végétale ou bactérienne comprenant la molécule d'acide nucléique de la revendication 2 ou le vecteur de la revendication 3.

5. Polypeptide isolé présentant une activité antiparasitaire, choisi dans le groupe comprenant:

- a) un polypeptide comprenant la séquence d'acides aminés de Séquence n° 2, 4 ou 6;
- b) un polypeptide comprenant une séquence d'acides aminés présentant au moins 80% d'identité de séquence avec la séquence d'acides aminés de Séquence n° 2, 4 ou 6, ledit polypeptide possédant une activité antiparasitaire;
- c) un polypeptide codé par la séquence de nucléotides de Séquence n° 1, 3 ou 5;
- d) un polypeptide codé par la séquence de nucléotides qui est identique au moins à 80% à la séquence de nucléotides de Séquence n° 1, 3 ou 5, ledit polypeptide possédant une activité antiparasitaire; et
- e) un polypeptide codé par la séquence de nucléotides de l'endotoxine delta de l'insert d'ADN du plasmide déposé sous le Numéro d'enregistrement NRRL B-30805, NRRL B-30809 ou NRRL B-30808.

6. Polypeptide de la revendication 5 comprenant en outre des séquences d'acides aminés hétérologues.

7. Composition comprenant le polypeptide de la revendication 5, ladite composition comprenant facultativement une poudre, poudre pour poudrage, des pastilles, des granules, un aérosol, une émulsion, un colloïde et une solution, et ladite composition étant préparée par dessiccation, lyophilisation, homogénéisation, extraction, filtration, centrifugation, sédimentation ou concentration d'une culture de la cellule hôte bactérienne de la revendication 4.

8. Composition de la revendication 7, comprenant d'environ 1% à environ 99% en poids dudit polypeptide.

9. Procédé d'éradication ou de contrôle d'une population de parasites lepidoptères, coleoptères ou nématodes, comprenant le fait de mettre en contact ladite population, ou de nourrir lesdits parasites, avec une quantité efficace du point de vue antiparasitaire d'un polypeptide de la revendication 5.

10. Procédé de production d'un polypeptide à activité antiparasitaire, comprenant le fait de cultiver la cellule hôte bactérienne de la revendication 4 en conditions dans lesquelles une molécule d'acide nucléique codant le polypeptide est exprimée, ledit polypeptide étant choisi dans le groupe comprenant:

- a) un polypeptide comprenant la séquence d'acides aminés de Séquence n° 2, 4 ou 6;
- b) un polypeptide codé par la séquence de nucléotides de Séquence n° 1, 3 ou 5;
- c) un polypeptide comprenant une séquence d'acides aminés présentant au moins 80% d'identité de séquence avec un polypeptide portant la séquence d'acides aminés de Séquence n° 2, 4 ou 6, ledit polypeptide possédant une activité antiparasitaire;
- d) un polypeptide codé par une molécule d'acide nucléique comprenant une séquence de nucléotides présentant au moins 80% d'identité de séquence avec la séquence d'acide nucléique de Séquence n° 1, 3 ou 5, ledit polypeptide possédant une activité antiparasitaire; et
- e) un polypeptide codé par la séquence de nucléotides de l'endotoxine delta de l'insert d'ADN du plasmide déposé sous le Numéro d'enregistrement NRRL B-30805, NRRL B-30809 ou NRRL B-30808.

11. Plante présentant un assemblage d'ADN incorporé de manière stable dans son génome, comprenant une séquence de nucléotides qui code une protéine possédant une activité antiparasitaire, ladite séquence de nucléotides étant choisie dans le groupe comprenant:

- a) la séquence de nucléotides de Séquence n° 1, 3 ou 5;
- b) une séquence de nucléotides présentant au moins 80% d'identité de séquence avec la séquence de nucléotides de Séquence n° 1, 3 ou 5, ladite séquence de nucléotides codant un polypeptide possédant une activité antiparasitaire;
- c) une séquence de nucléotides qui code un polypeptide comprenant la séquence d'acides aminés de Séquence n° 2, 4 ou 6;
- d) une séquence de nucléotides qui code un polypeptide présentant au moins 80% d'identité de séquence d'acides aminés avec la séquence d'acides aminés de Séquence n° 2, 4 ou 6, ladite séquence de nucléotides codant un polypeptide possédant une activité antiparasitaire; et
- e) la séquence de nucléotides de l'endotoxine delta de l'insert d'ADN du plasmide déposé sous le Numéro d'enregistrement NRRL B-30805, NRRL B-30809 ou NRRL B-30808, ou une séquence complémentaire de celle-ci;

ladite séquence de nucléotides étant liée opérationnellement à un promoteur qui commande l'expression d'une séquence codante dans une cellule végétale.

12. Plante de la revendication 11, ladite plante étant choisie dans le groupe comprenant le maïs, le sorgho, le blé, le chou, le tournesol, la tomate, les crucifères, les poivres, la pomme de terre, le coton, le riz, le soja, la betterave à sucre, la canne à sucre, le tabac, l'orge et la navette oléagineuse.

13. Graine transformée de la plante de la revendication 12, ladite graine comprenant, incorporé dans son génome, un assemblage tel que défini en revendication 11.

14. Procédé de protection d'une plante contre un parasite, comprenant le fait d'introduire dans ladite plante, ou dans une cellule de ladite plante, au moins un vecteur d'expression comprenant une séquence de nucléotides qui code un polypeptide antiparasitaire, ladite séquence de nucléotides étant choisie dans le groupe comprenant:

- a) une molécule d'acide nucléique comprenant la séquence de nucléotides de Séquence n° 1, 3 ou 5;
- b) une molécule d'acide nucléique comprenant une séquence de nucléotides présentant au moins 80% d'identité de séquence avec une séquence de nucléotides de Séquence n° 1, 3 ou 5, ladite séquence de nucléotides codant un polypeptide possédant une activité antiparasitaire;
- c) une molécule d'acide nucléique qui code un polypeptide comprenant la séquence d'acides aminés de Séquence n° 2, 4 ou 6;
- d) une molécule d'acide nucléique qui code un polypeptide présentant au moins 80% d'identité de séquence avec la séquence d'acides aminés de Séquence n° 2, 4 ou 6, ladite séquence de nucléotides codant un polypeptide présentant une activité antiparasitaire; et
- e) la séquence de nucléotides de l'endotoxine delta de l'insert d'ADN du plasmide déposé sous le Numéro d'enregistrement NRRL B-30805, NRRL B-30809, NRRL B-30808, ou une séquence complémentaire de celle-ci.

15. Procédé selon la revendication 14, ladite plante produisant un polypeptide antiparasitaire possédant une activité antiparasitaire contre un parasite lepidoptère, coleoptère ou nématode.

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*      20      *      40      *      60      *      80
axmi018 tr : HTQNHFSFSDNTSSSTGVSTLESSLIPYVNYATDQFNSNKNWEDALKKLEKFYSGDLTQDAIDIFLGDSGFDYLSLVNVIFSI : 83
axmi020 tr : HTQNHFSFSDNTSSSTGVSTLESSLIPYVNYATDQFNSNKNWEDALKKLEKFYSGDLTQDAIDIFLGDSGFDYLSLVNVIFSI : 83
axmi021 : HTQNHFSFSDNTSSSTGVSTLESSLIPYVNYATDQFNSNKNWEDALKKLEKFYSGDLTQDAIDIFLGDSGFDYLSLVNVIFSI : 83
HTQNHFSFSDNTSSSTGVSTLESSLIPYVNYATDQFNSNKNWEDALKKLEKFYSGDLTQDAIDIFLGDSGFDYLSLVNVIFSI

*      100     *      120     *      140     *      160
axmi018 tr : AGSFIPYVGALVPIINLLFGSESKPDVFEQMRARIEALIHKELSAHVQTLKAEIKGLKDTGDLVQKDVNAVAGRITNGPTPPS : 166
axmi020 tr : AGSFIPYVGALVPIINLLFGSESKPDVFEQMRARIEALIHKELSAHVQTLKAEIKGLKDTGDLVQKDVNAVAGRITNGPTPPS : 166
axmi021 : AGSFIPYVGALVPIINLLFGSESKPDVFEQMRARIEALIHKELSAHVQTLKAEIKGLKDTGDLVQKDVNAVAGRITNGPTPPS : 166
AGSFIPYVGALVPIINLLFGSESKPDVFEQMRARIEALIHKELSAHVQTLKAEIKGLKDTGDLVQKDVNAVAGRITNGPTPPS

*      180     *      200     *      220     *      240
axmi018 tr : FDSNTDALKAE LRSQITATNTL FVQRHPQFAIEGYEEITLPLHTIAASHHLIFLKDVCHEGAEWGIANNTLTNYQGQLQDCIR : 249
axmi020 tr : FDSNTDALKAE LRSQITATNTL FVQRHPQFAIEGYEEITLPLHTIAASHHLIFLKDVCHEGAEWGIANNTLTNYQGQLQDCIR : 249
axmi021 : FDSNTDALKAE LRSQITATNTL FVQRHPQFAIEGYEEITLPLHTIAASHHLIFLKDVCHEGAEWGIANNTLTNYQGQLQDCIR : 249
FDSNTDALKAE LRSQITATNTL FVQRHPQFAIEGYEEITLPLHTIAASHHLIFLKDVCHEGAEWGIANNTLTNYQGQLQDCIR

*      260     *      280     *      300     *      320     *
axmi018 tr : EYSNKAYSHFNIGLQRAKNNGNHNNVNNVYIRTHKLNALDTVAQWPLDKVITYPLDITLQQTGRGIFSDLSGRGGTQSNRYRD : 332
axmi020 tr : EYSNKAYSHFNIGLQRAKNNGNHNNVNNVYIRTHKLNALDTVAQWPLDKVITYPLDITLQQTGRGIFSDLSGRGGTQSNRYRD : 332
axmi021 : EYSNKAYSHFNIGLQRAKNNGNHNNVNNVYIRTHKLNALDTVAQWPLDKVITYPLDITLQQTGRGIFSDLSGRGGTQSNRYRD : 332
EYSNKAYSHFNIGLQRAKNNGNHNNVNNVYIRTHKLNALDTVAQWPLDKVITYPLDITLQQTGRGIFSDLSGRGGTQSNRYRD

*      340     *      360     *      380     *      400     *
axmi018 tr : YDAVOGYAPpFVGFDtKLNWVNDFGYKDLTAIQTFtGDRIDSIWQSFKYNSGEPFLTNLGNKGpGNNPVIIPNSRDNPiISAKG : 415
axmi020 tr : YDAVOGYAPpFVGFDtKLNWVNDFGYKDLTAIQTFtGDRIDSIWQSFKYNSGEPFLTNLGNKGpGNNPVIIPNSRDNPiISAKG : 415
axmi021 : YDAVOGYAPpFVGFDtKLNWVNDFGYKDLTAIQTFtGDRIDSIWQSFKYNSGEPFLTNLGNKGpGNNPVIIPNSRDNPiISAKG : 415
YDAVOGYAPpFVGFDtKLNWVNDFGYKDLTAIQTFtGDRIDSIWQSFKYNSGEPFLTNLGNKGpGNNPVIIPNSRDNPiISAKG

*      420     *      440     *      460     *      480     *      5
axmi018 tr : SRPSANYVGHNFQRAKTKVVtNGYVtIPNDNYTVPAGHKLGWISALHDELDNANNADLVSVVVKNDIFQENIIGSIKTVTTDD : 498
axmi020 tr : SRPSANYVGHNFQRAKTKVVtNGYVtIPNDNYTVPAGHKLGWISALHDELDNANNADLVSVVVKNDIFQENIIGSIKTVTTDD : 498
axmi021 : SRPSANYVGHNFQRAKTKVVtNGYVtIPNDNYTVPAGHKLGWISALHDELDNANNADLVSVVVKNDIFQENIIGSIKTVTTDD : 498
SRPSANYVGHNFQRAKTKVVtNGYVtIPNDNYTVPAGHKLGWISALHDELDNANNADLVSVVVKNDIFQENIIGSIKTVTTDD

*      600     *      620     *      640     *      660
axmi018 tr : TPLPNTSSSTSTQTKVtIQGLQKQYQLINGPcLDLTAGSHTFGImLTANaSONVFIDRIEFVPIa3tepvtiPntPiktytnp : 664
axmi020 tr : TPLPNTSSSTSTQTKVtIQGLQKQYQLINGPcLDLTAGSHTFGImLTANaSONVFIDRIEFVPIa3tepvtiPntPiktytnp : 663
axmi021 : TPLPNTSSSTSTQTKVtIQGLQKQYQLINGPcLDLTAGSHTFGImLTANaSONVFIDRIEFVPIa3tepvtiPntPiktytnp : 664
TPLPNTSSSTSTQTKVtIQGLQKQYQLINGPcLDLTAGSHTFGImLTANaSONVFIDRIEFVPIa3tepvtiPntPiktytnp

*      680     *      700
axmi018 tr : pnpqqv6UTagpGilgd6vN g n : 700
axmi020 tr : ---RKTvutSSSTGTAFSvEMHtGpGHRNFNDRIEFLOKG : 700
axmi021 : pnpqqv6UTagpGilgd6vN g n : 693
pnpqqv6UTagpGilgd6vN g n

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FIG. 1

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*      20      40      60      80
axmi018 tr : -HTQNHSFSDNTSSSTGVST--LESSLIPVNVYATDFNSNKNVEDALKKLLEKFYSGDLTODAIDIFLGDSCGFDYLSLVNVI : 80
cry12Aa tr : MATLNEVYVNVYVNLSSDAF--QQLDTTGKSKSYDEHIKAFERKUKKGAAGK-----DLLDVAVTYITTTGIDPLNVKGV : 74
cry21Aa tr : MTNPTILYPSYHNVLAHPIR--LDSFFDPYVETFKDLKGAVEEFKGTGYHDP-----LKQHLQTAUDTSONGTVDYLAATKAS : 76
cry21Ba1 t : MADLTLEYPSYHNVLAHPIR--LDSIFDPYIDIFNALKGGUEEFKGTGYKDP-----LEQHLKTAUNASONGTVDYLAATKAS : 76
cry5Aa tr : MAILNELYPSVYPNVLAYTP--PSFLPDAGTOATPADLTAYEQLLKNLEKGINAGTYSKAIADVLKGFIDDTINYQTYVNIC : 81
cry5Ab tr : MAILNELYPSVYPNVLAYTP--PSFLPDAGTOATPADLTAYEQLLKNLEKGINAGTYSKAIADVLKGFIDDTINYQTYVNIC : 81
cry5Ba tr : MAILNELYPSVYPNVLAYTP--PSFLPDAGTOATPADLTAYEQLLKNLEKGINAGTYSKAIADVLKGFIDDTINYQTYVNIC : 81
cry1Ac tr : MATINELYPSVYPNVLAHPK--EVDDPYPSNLLKGIQEGWEEUGTKGOKKL-----FEDHLTAUNLYKTGKLDYFALTKAS : 76
cry1Ba tr : -----MDNNP--NINECIPYCNLSNPEVEVLG-----G-----ERITGYTPIDISLSLT : 43
cry1Ca tr : -----HTSNRKNENEIINAVSNHSAQMDLLPDARIEDSLCIAE-----GNN--IDPFVSASTVQTGGINIAG : 59
cry1Ca tr : -----MEEN--NQNCIPYCNLSNPEE-----VLADG-----ERISTGNSSIDISLSLV : 42

*      100      120      140      160
axmi018 tr : FSIAGSFIPVYVGVNLLFGSES-----K-----PDVSEQRARDEALTHKELSAHVQTKAEHKKKKTGDLTKKD : 151
cry12Aa tr : LSVMT-LIPEVGTMAASAATIVSFDPKPIFGDKPN-----AKNISEELKPCHEALHOQDTINYQDAINQKFKDSQKKTINLVTA : 153
cry21Aa tr : ISFHG-LIPGADAVVPPFNHFVDFPFKLFGRGSQONAQOQFELIIEKVKELVDQDFRNFILNNDLNDGQOTLASHQND : 158
cry21Ba1 t : ISFHG-LIPGADAVVPPFNHFVDFPFKLFGRGSQONAQOQFELIIEKVKELVDQDFRNFILNNDLNDGQOTLASHQND : 158
cry5Aa tr : LSVMTLAVPEIGITPFFHGLFFAAFNKHDAPPPPN-----AKDISEANKPAHQEHDRTLTADQOTFLNGEISGONLAARQST : 161
cry5Ab tr : LSVMTLAVPEIGITPFFHGLFFAAFNKHDAPPPPN-----AKDISEANKPAHQEHDRTLTADQOTFLNGEISGONLAARQST : 161
cry5Ba tr : ISFHG-FIPGAEAAVPPFNHFVDFPFKLFGANTEG-KDQQLNANLHDAFNKHNKFLSYNLSTLNKTHESGONLGLQNA : 157
cry1Ac tr : QFSESEFVSGAFVVLGLDITUGIFG-----PSQDAFLVQHEQLINQRIEEFARNQATSRSEESNLYQIAES : 113
cry1Ba tr : RIAGVLGVBFAGQASFYSLVGEIUPFR-----GRDQEIFLEHVEQLINQOTENARNALARQOQIGDSFRANQOS : 132
cry1Ca tr : QFISVNFVGGGFLVGLDITUGIFG-----PSQDAFLVQHEQLINQRIEEFARNAAIAMEGEGNNFNINIVEA : 112

*      180      200      220      240
axmi018 tr : UNAVAGRTNGTPTPSFDSN-----TDALKAERISQITATNLFVQRHQAIEGY-----FEETPLPHHTIA : 212
cry12Aa tr : MDNDYVTAK-----TQENLNSILTSIDISIFIEGYETGG-----LEYNAHV : 196
cry21Aa tr : QOIAICQEQGLH-LDQTPACTPT--TDHLISVRESFKDARTIETALFHKNPMLSTNDNTPDFNSDTVLTLTPRTTG : 237
cry21Ba1 t : QOIAICQEQGLI-LDEKHPCTPT--KNHLVSQKESFKNARSIEITVLEHKNPHT--NNKTPDFNSDTVLTLTPRTTA : 235
cry5Aa tr : DDIDQSHGG-----FNKQDSGLIKKFDDEVLSNSRYTDRLP--VFITDNTADRTLGLUPYAIL : 219
cry5Ab tr : DDIDQSHGG-----FNKQDSGLIKKFDDEVLSNSRYTDRLP--VFITDNTADRTLGLUPYAIL : 219
cry5Ba tr : QOIAICQEQGLH-LDQTPACTPT--TDHLISVRESFKDARTIETALFHKNPMLSTNDNTPDFNSDTVLTLTPRTTG : 237
cry1Ac tr : FREWEADPTNP-----ALREEIRIQFNDHNSALTTAIFLFAVQNYQVP-----LESYVQA : 164
cry1Ba tr : MEDULENRDDR-----TRSVYVQVIALELDFLNAELFAIRNQE-----VPLIMVQA : 183
cry1Ca tr : FKEWEEDPNNP-----ATRTRVDRFRILDGLLERDIESERISGFVFP-----LESYVQA : 163

*      260      280      300      320
axmi018 tr : ASHHLLELDVCEHGAELGIAN-----TTTNYQGGHODCHRESENKAYSFNIGFORAKN--NGNNHNVNVNITKKNALD : 290
cry12Aa tr : ANAHLELLEDAVNAELKGF-----SKDEVTHKKYKKTTHNVHEAVIKAFNLGSDKFKS--LDVNSYNNKANNIEGHENVL : 274
cry21Aa tr : ANAHLEHGGYVQFAERKKS--VNYDESPINOTKVDNORRHQDFTTMTTTEKFKPTLNP--SNKESVNNKNNRVSSEHLQSLD : 318
cry21Ba1 t : ANAHLEHGGYVQFAERKKS--VDYDEAFINOTKADNORRHQDFTTMTTTEKFKPTLS--NKKSEINTNKRVSSEHLQSLD : 315
cry5Aa tr : ASHHLLELDITKGPITDSKINFPTDADISFKTDKNNNKLSEKTLVDVQKGLASGYTPSDLESFAKKOKIEIINTHCLD : 302
cry5Ab tr : ASHHLLELDITKGPITDSKINFPTDADISFKTDKNNNKLSEKTLVDVQKGLASGYTPSDLESFAKKOKIEIINTHCLD : 302
cry5Ba tr : ANAHLEHGGYVQFAERKKS--VNYDESPINOTKVDNORRHQDFTTMTTTEKFKPTLNP--SNKESVNNKNNRVSSEHLQSLD : 318
cry1Ac tr : ANAHLEHGGYVQFAERKKS--VDYDEAFINOTKADNORRHQDFTTMTTTEKFKPTLS--NKKSEINTNKRVSSEHLQSLD : 315
cry1Ba tr : ANAHLEHGGYVQFAERKKS--VNYDESPINOTKVDNORRHQDFTTMTTTEKFKPTLNP--SNKESVNNKNNRVSSEHLQSLD : 318
cry1Ca tr : ANAHLEHGGYVQFAERKKS--VDYDEAFINOTKADNORRHQDFTTMTTTEKFKPTLS--NKKSEINTNKRVSSEHLQSLD : 315

*      340      360      380      400
axmi018 tr : TVLQCHILDKVTIEL-----DTTLQTRCTFSDLS--GR-----GGTCSNYRYDYDAVQC-----YAPPFVG--FDTKLN : 351
cry12Aa tr : TVLQCHILDKVTIEL-----EVELEFTRTIESPIYQVPKN--MONTSESIYPSDLFHYOGDLVKLEFSTRITDNDGLAKIFT : 349
cry21Aa tr : IATUTPILDNVNVYS--NV--DQOLDQTLVFSVAGVMEGN--DNITSNIIDVLTPINT-----GIGFOES--SDLRKFTY : 387
cry21Ba1 t : IATUTPILDNVNVYS--NV--EQOLDQTLVFSVNLVGFEGN--DDISTYTRSIHNYSK-----GDTFGDVNSAIQSLRY : 385
cry5Aa tr : FARLPFTIPDPLVETGSG--DLSLQKTRRLSPPIRTAD--GLTLNNTSITDTSNUPN-----YENGNGAFPNPKERIL : 373
cry5Ab tr : FARLPFTIPDPLVETGSG--DLSLQKTRRLSPPIRTAD--GLTLNNTSITDTSNUPN-----YENGNGAFPNPKERIL : 373
cry5Ba tr : IATUTPILDNVNVYS--NV--EQOLDQTLVFSVNLVGFEGN--DDISTYTRSIHNYSK-----GDTFGDVNSAIQSLRY : 385
cry1Ac tr : TVLQCHILDKVTIEL-----DTTLQTRCTFSDLS--GR-----GGTCSNYRYDYDAVQC-----YAPPFVG--FDTKLN : 351
cry1Ba tr : TVLQCHILDKVTIEL-----DTTLQTRCTFSDLS--GR-----GGTCSNYRYDYDAVQC-----YAPPFVG--FDTKLN : 351
cry1Ca tr : IATUTPILDNVNVYS--NV--EQOLDQTLVFSVNLVGFEGN--DDISTYTRSIHNYSK-----GDTFGDVNSAIQSLRY : 385

*      420      440      460      480
axmi018 tr : VV-----NDFGYKDLTAIQFTT--GDRIDSIQSFKYNSGEFF--LTLNGNGKPGNNPVIPN-----SRDNPIISAKGRSP : 418
cry12Aa tr : GI-----RNTFYKSPNTHETIYH--VDFSNTYQSSGNISRGSSN--FPIIDLNNPIISTCIRNSFYK-----AIAGSSVLVDFKDG : 420
cry21Aa tr : PR-----IELQSNQFHGYVNS--KSVEHCYSGLKLYKNKT--ITAGVSNIDESNQNKNHNYGP-----VINSFIDTQVNSQ : 458
cry21Ba1 t : PR-----LELSKVQFYTHDQRS--NGVRHCYTSGFNLTFFDNDS--SMSAKQDESATADS-----P-----PLTARIKNNQANSQ : 450
cry5Aa tr : KO-----FKLYPSURAGQYG--GLLOPYLWAEVQDSVET--RLYGQLPAVDPOAG--PNYVSI-----DSSNPIIQIIMDTU : 440
cry5Ab tr : KO-----FKLYPSURAGQYG--GLLOPYLWAEVQDSVET--RLYGQLPAVDPOAG--PNYVSI-----DSSNPIIQIIMDTU : 440
cry5Ba tr : SR-----KELQQLDIATYSTN--NSMNCHPYGLRLSYTDGS--RYDYGDNDQPFDTTSMNNYCHN-----SYTAPITLVNARHL : 462
cry1Ac tr : G-----YYVUSGHQINASPVGSGPEFTPLYGTMGNAAPOQRIIVAQLGQGVYRTLSSTLYRR-----PFNIGCTNNQOLSVL : 383
cry1Ba tr : USNTRHMTVURGHTIQSRP--IGGGLNTSTHGAINTSINPVTLRFASRDVYRTESYAGVLLUGIYLEPIHGVETVRFVTFNP : 419
cry1Ca tr : VG-----RNFYUGGHRVSSLI--GGGNITSPYIGREANQEP--RSFTFNGPVFRTLNNPFLRL-----QOPUPAPPFILRGV : 386

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FIG. 2A

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      00          *          520          *          540          *          560          *          580
axmi018 tr : S-----ANYVGHNFQRANKTMVSNQYVVPNDN-----YTVPAHGKLGWISALHDELDNAN-N-----ADLV : 473
cry12Aa tr : T-----QGYAFAQAPTGGAUDHSFIESDG-----APEGHKLNVLYTSPGDLRDFIN-----VYTL : 471
cry21Aa tr : N-----SQYLDLNSVHVNGG-QKYTGCSPLSSNGN-----SNMAALPNQKINVIYSVQSNCKPEK-----HADTYRKUGY : 522
cry21Ba1 t : N-----SQYVDYSSINIDN--QGGGGCSAFPYSYQ-----SNNPILPNQKINVFYYPGSSAHPIDPH-----TTDPDTWFKLGY : 516
cry5Aa tru : K-----TPPGGASGUNTNLHRGSVSGLSFQORDGT-----RLSAGHGCGFADTIYSLPATHYLSYLYGTPYQTSDNYSGHVGA : 514
cry5Ab tru : K-----TPPGGASGUNTNLHRGSVSGLSFQORDGT-----RLSAGHGCGFADTIYSLPATHYLSYLYGTPYQTSDNYSGHVGA : 514
cry5Ba tru : YNAKGLQNVESLVVSTVNGGSGCICDAWYNILRPPQTSKNESRPDQKINVLYPITETVNNKGTG-----G-----NLGV : 532
cry1Ac tru : DGTEFAYGTSSNLPSAVYRKSGTVDSLDETPPONN-----NVPPRQGFSHRLSHVSHFRSGFSN-----SSVSLT : 448
cry1Ba tru : QN-ISRGTANYSQPYESPLQLKDSETEPPETTE-----RPNYESYSHRLSHIGIILQSR-----M : 476
cry1Ca tru : EG----VEFSTPTNSFTYRGRGTVDSLTEPPEDN-----SVPPREGYSHRLCHATFVQRSG-----TPFLT : 444

      *          600          *          620          *          640          *          660
axmi018 tr : MSVVVKNDIFQENIGSTKTVTTDDGTTENRQDITGIDADRHHTRS-----TKRHELEFINGINGSHLSSTND-----QLYYTIN : 549
cry12Aa tr : LSTPTINELSTEKIKGFPAEKG-----YDKNOGIMRYYGK-----PEYINGAQPVNLENQO-----TLIFEFH : 529
cry21Aa tr : MSSHIPYDLVPENIGDIDPDTKQP-----SLLKGFPAEKGYGDS-----IAYVSEPLNGANAVKLTSYQ-----VLQHEHT : 590
cry21Ba1 t : MSSHIPYDLTPQWIGELDQDTKQP-----SLIKGFPAEKGYGGS-----IEYVSEPLNGANAARKLTNLQ-----ILVHOYT : 584
cry5Aa tru : MGVSTPQEAATLPNIGOPDEQG-----NVSTHGFPERASYG-----GTVVKEMLNGANAKLSPGQ-----SIGIPIT : 578
cry5Ab tru : MGVSTPQEAATLPNIGOPDEQG-----NVSTHGFPERASYG-----GTVVKEMLNGANAKLSPGQ-----SIGIPIT : 578
cry5Ba tru : TSAYVPHELVPENIGDYNADTKLP-----LTOKGFPERASYGSEYNNRGTSLVREMLNGANAVKLENSQ-----SVGIQIT : 604
cry1Ac tru : RAPNFSUIHRSAEFNNILASDS-----HTQIRAVMGNFLFNG-SMISGPGFTGCDLVRNLSSGNNIQNGYIEVPIH : 519
cry1Ba tru : NVPVYSWTHRSADRTNITGPNR-----HTQIRAVMASELPQGTTVVRGPGFTGCDILRRNTTGGFG-----PIRVTIN : 544
cry1Ca tru : TGVVFSWTHRSATENTLDPER-----LNQIELVGRFVWGGSMTITGPGFTGCDILRRNTTGGFG-----SLQVNDN : 512
      p K          G          6

      *          680          *          700          *          720          *          740
axmi018 tr : PIVS-QRYVLSYRVVATTSAESLDIUIDGYKRG-----TPNPNTSSTSTQTKRVIIQGLQCKYQLINGPTEDLTAGSHT-- : 622
cry12Aa tr : ASKQ-ACQYTIIRMAST--QCTKGYFLDNQEL-----QTNIPTSHNGYMTG-----NIGENYDLMTIGSYTITEGNHT-- : 596
cry21Aa tr : NOTK-QKYVRIIRMAATGGDTAASITPHIIGPSG-----NDITNEGHNFSSVSSRNKMFVQGNNGKVVNLITDSIELPS-- : 663
cry21Ba1 t : NLTK-QRYVIELRHAKNDTTASITPHIIGPNN-----QDMINHSPDIPPRSN-NKMFVQGNNGKVVLDTLVDSIELPS-- : 656
cry5Aa tru : NVTE-GEYQIECRHASN--DNTNGFENVDTGGA-----NPIFQQINFASITDN--NTGVQGANGVVVKSTATTDNSTF-E : 648
cry5Ab tru : NVTE-GEYQIECRHASN--DNTNGFENVDTGGA-----NPIFQQINFASITDN--NTGVQGANGVVVKSTATTDNSTF-E : 649
cry5Ba tru : NOTK-QKYVIELRHASKG--DNNVYFNVDLSE-----NPFNRNSISFGSTESS--VVGQGNNGKVIIRKSTITVEIPAG-- : 672
cry1Ac tru : FPSHSTRYRVVRBASVTPIHLMNMGNSSIFS-----NTUPATATSLDNLOSSDFGYFESANAFTSSLGNIVGVNRFE-- : 593
cry1Ba tru : GPLK-QRYRIGFRHASTVDFFVSRGGTTVNN-----FRFLRTHNSGDELKYGNFVRRRAFTTPTFTTQIQDIIRTSIQGL : 619
cry1Ca tru : SPIN-QRYRLFRHASSRDARVITLTGAASTGVGGQVSVNHPLOKTHEIGENTSRTRRYTDFSNPFSFRANPDIIGISEQ-- : 592
      Y 6r RyA3

      *          760          *
axmi018 tr : ----FCIHLTANASQWVIDRDEEVE-- : 644
cry12Aa tr : ----TQIQHNDKNGHVLDRDEEVEKD : 618
cry21Aa tr : G-QQTILITONTN--SODLELDRDEEISLP : 689
cry21Ba1 t : G-QLTILITONINP--DODLELDRDEEVIP : 683
cry5Aa tru : IPAKTINVHLTNQSSDVELEDRDEEIP-- : 675
cry5Ab tru : IPAKTINVHLTNQSSDVELEDRDEEIP-- : 676
cry5Ba tru : ----SFYMHITNQSSDLELDRDEEIP-- : 695
cry1Ac tru : ----G-TAGVIDRDEEIPVT : 609
cry1Ba tru : S-----G-NGEVIDRDEEIPVT : 636
cry1Ca tru : ----PLFGAGSISSELEVIDRDEEILAD : 616
      6 6D4iEf6p

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FIG. 2B

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axmi020 tr : --HTONHSTSDNTSSSTGVST--LESSLIPYNNVYATDQNSNKWEDALKKLEKFYSGDLTQDAIDIFLGDSCGFYLSLVNVI : 80
cry12Aa tr : MATLNEVYPVNNVNLSSDAF--QQLDTTGFKSKYDEHKAPEKKUKKGAAGK-----DLLDVAVTYITTTGEIDPLNVIKGV : 74
cry21Aa tr : MTNPTILYPSYHNVLAHPIR--LDSFFDPFVETFKDLKGAVEEFKGTGYMDP-----LKOHLQIAUDTSONGTVDYLAATKAS : 76
cry21Ba1 t : MADLTELVPYHNVLAHPIR--LDSIFDPFIDIFNALKGGVEEFKGTGYMDP-----LEOHLKTAUNASONGTIDYLAATKAS : 76
cry5Aa tr : MAILNELYPSVPYNNVLAHTP--PSFLPDAGTQATPADLTAYEQLLKXNLEKGINAGTYSKATADVLKGFIDDTINYQTYVNIG : 81
cry5Ab tr : MAILNELYPSVPYNNVLAHTP--PSFLPDAGTQATPADLTAYEQLLKXNLEKGINAGTYSKATADVLKGFIDDTINYQTYVNIG : 81
cry5Ba tr : MATINELYVPYNNVLAHPIR--EVDPPYSUNLLKQIQEGVEUGKTCQKKL-----FEDHLTIAUNLYKTGKLDYFALTKAS : 76
cry1Ac tr : -----HDNNP--NINECIPYNNCLSNPEVEVLG-----G-----ERIECTGYTPIDISLSLT : 43
cry1Ba tr : -----HTSNRKVENEIINAVSNHSAQMDLLPDARIEDSLCTAE-----GNN--IDPFVSASTVQTCGNIAG : 59
cry1Ca tr : -----NEEN--NQNCIPYNNCLSNPEE-----VLLDG-----ERISTGNSSIDISLSLV : 42

axmi020 tr : FSHAGSFIPYVGLVPIENLLFGSES-----R-----PDVFEQHRARIEALNHKEL'SADHVQTLKAEHKEKLDGTGDLTKQD : 151
cry12Aa tr : LSNHT-LIPEVGTMAAASSTIVSFHUPKIFGDKPN--AKNIFEELKPCIEALQOODITNYQDAINQKKFDSLQKTLNLTVA : 153
cry21Aa tr : ISHFG-LIPGADAVVPPNNHFVDFHPPKLFGRGSGQNAQAQOFFELTIEKVKELVQEDFRNFILNNLLNYSQCHQATLSHSOND : 158
cry21Ba1 t : ISHFG-LIPDADAVVPPNNHFVDFHPPKLFGRGSGQNSQAQOFFELTIEKVKELVQEDFRNFILNNLLNYSQCHQATLSHSOND : 158
cry5Aa tr : LSNHTLAVPEIGIFTPFGLFFAANKHDAPPPPN--AKDIFEAKHPAQEHIDRTLTADEQTFNGEISGLONLAARYQST : 161
cry5Ab tr : LSNHTLAVPEIGIFTPFGLFFAANKHDAPPPPN--AKDIFEAKHPAQEHIDRTLTADEQTFNGEISGLONLAARYQST : 161
cry5Ba tr : ISHFG-FIPGAAAVPPNNHFVDFHPPKLFAGANTEG-KDQOLNATINDANKVONKFLSYNLSLTNKTIEGLQNLGLGFONA : 157
cry1Ac tr : OFLSEFVPGAGFVLGLDIDIWGIFG-----PSQDAFLVQIEQLNQRIEFAFNQAISRAEGLSNLYQIVAES : 113
cry1Ba tr : RINGVLQVFFAGQLASEYSFLVGEHUPR-----GRDQEIFLEHVEQLNQOITENARNTLARNGLGDSFRAYQOS : 132
cry1Ca tr : OFLSENFVGGGFLVGLDIDFVUGIFG-----PSQDAFLVQIEQLNRIEFAFNQAIAIANEGLGNFNLTVEA : 112

axmi020 tr : VNAVAGRNGTTPSFDSN-----TDALKAEFRSQITATNLFVORHQAIEGY-----EETTPHHTIA : 212
cry12Aa tr : DNNNDYVTAK-----TCQENLNSILTQDISIPHEGYETGG-----CRYVANV : 196
cry21Aa tr : VOIAICQGEQGLH-LDQTPTACTPT--TDHLISURESEKADARTIETALHKNPHLSTNDNTPDFNSDVLTLTPMTTG : 237
cry21Ba1 t : VOIAICQGEQGLI-LDEKHPPCTPT--QNHLSVYKESFKNARSIEYTVLHKNPHL--NNKTPDFNSDVLTLTPMTTA : 235
cry5Aa tr : HDDIQSHGG-----FNKVDSEGLKKKFEVLSNSGYTDRLP--VFITDNTADRTLGLGQRYAIL : 219
cry5Ab tr : HDDIQSHGG-----FNKVDSEGLKKKFEVLSNSGYTDRLP--VFITDNTADRTLGLGQRYAIL : 219
cry5Ba tr : HQVAICQGSTPERVNFONCTPCPNPQCKDDLDRASRTPTANSQFQHLHKNPUSD-ENSTQEFKRTSVELTPMTTV : 239
cry1Ac tr : PREUEADPTNE-----ALREERQCFNDHNSALTTAHLAVQNYQVP-----LDMVYQA : 164
cry1Ba tr : BEDULENRDDAR-----TRSVLYTQYIALEDFLNAHLAIRNOE-----VPLDMVYQA : 163
cry1Ca tr : FKEUEEDFNNP-----ATRRVYIDRFRILDGLLDRHLSRISGFVFP-----LDMVYQA : 163

axmi020 tr : ASHHTLPLKDVCEHGAEGIAN-----TTITNVQGGQODCQREHFNKAYSMNIGHVORAKN-NGNNHNNNNNNNITKKNALD : 290
cry12Aa tr : ANAHLIGERDADVNAEKLGF-----SDKEVDTKKKYMKRTDHHHEAVIKALNGLDQFKS-LDVSYNKKKANNAGREHVL : 274
cry21Aa tr : ATNHLHGGGYLQFAERLKS-VNYDESPINQTVDLQRRQDSTTVSTTEKFKPILNP-SNKESVNKYNNVNSHGLQSL : 318
cry21Ba1 t : ATNHLHGGGYLQFAERLKS-VDYDEAPINQTVDLQRRQDSTTVSTTEKFKPILNS--NKKSSINTYNNVNSHGLQSL : 315
cry5Aa tr : ASHHTLPLRDLITKGPITDSKINFTPDAISFKTDKNNKLSKTYLVDVQKGLASGYTPSGLSEAKKQKVEIITHCLD : 302
cry5Ab tr : ASHHTLPLRDLITKGPITDSKINFTPDAISFKTDKNNKLSKTYLVDVQKGLASGYTPSGLSEAKKQKVEIITHCLD : 302
cry5Ba tr : ATNHLHGGGYLQFAERLKS-VNYDESPINQTVDLQRRQDSTTVSTTEKFKPILNP-SNKESVNKYNNVNSHGLQSL : 318
cry1Ac tr : ANAHLIGERDADVNAEKLGF-----SDKEVDTKKKYMKRTDHHHEAVIKALNGLDQFKS-LDVSYNKKKANNAGREHVL : 274
cry1Ba tr : ANAHLIGERDADVNAEKLGF-----SDKEVDTKKKYMKRTDHHHEAVIKALNGLDQFKS-LDVSYNKKKANNAGREHVL : 261
cry1Ca tr : ANAHLIGERDADVNAEKLGF-----SDKEVDTKKKYMKRTDHHHEAVIKALNGLDQFKS-LDVSYNKKKANNAGREHVL : 241

axmi020 tr : TVACUPLLDKVTYEL-----DITLQOTRGITFDLS--GR--GGTQSNRYRYDYAVQG-----YAPSFVG--FDTELN : 351
cry12Aa tr : LVNLWPPFDPDHYOK-----EVELEFTRTISPIYQVPKPN--MONTESHINPSDLFHYQGDVLKLEFSTRTDNDGLAKIFT : 349
cry21Aa tr : TARTUPLLDVNNVYS-NV-EHQDQTRLVFNENLVGPFEGN--DNITSNIIDVLPTINT-----GIGFQES--SDLRKFTY : 387
cry21Ba1 t : TARTUPLLDVNNVYS-NV-EHQDQTRLVFNENLVGPFEGN--DDISTYTRRSINNYK-----GDTPGDVNSAIOQLRY : 385
cry5Aa tr : FURLFPFDPDLVYTGSG-DLSLQKTRRLSPFFPIRTAD--GLTLNNTSIDTNSWPN-----YENGNGAFPNNKERIL : 373
cry5Ab tr : FURLFPFDPDLVYTGSG-DLSLQKTRRLSPFFPIRTAD--GLTLNNTSIDTNSWPN-----YENGNGAFPNNKERIL : 373
cry5Ba tr : TARTUPLLDVNNVYS-NV-EHQDQTRLVFNENLVGPFEGN--DNITSNIIDVLPTINT-----GIGFQES--SDLRKFTY : 387
cry1Ac tr : LVNLWPPFDPDHYOK-----EVELEFTRTISPIYQVPKPN--MONTESHINPSDLFHYQGDVLKLEFSTRTDNDGLAKIFT : 349
cry1Ba tr : LVNLWPPFDPDHYOK-----EVELEFTRTISPIYQVPKPN--MONTESHINPSDLFHYQGDVLKLEFSTRTDNDGLAKIFT : 311
cry1Ca tr : LVNLWPPFDPDHYOK-----EVELEFTRTISPIYQVPKPN--MONTESHINPSDLFHYQGDVLKLEFSTRTDNDGLAKIFT : 339

axmi020 tr : VV--NDFGKYKDLTAIQTTG--DRIDSUQSFYKNSGE--PFLTNLGNRGKNNPVI-PNSRDNPISAKGSRPS-- : 419
cry12Aa tr : GI--RNTEYKSPNTHETVHV--DFSNTQSSGNTISRGSSNPIDILNPIISTCIRNSF-VKAIACSSVLVNFKDGCT-- : 421
cry21Aa tr : PR--IELQSHQFHQVYNSK--SVEHCYSDGLKLVNKNITAGVSNIDESNQNNKNHY-GPVINSPTDINUNSON-- : 459
cry21Ba1 t : PR--LELSKVQFYTHDQRSN--GVRHCYTSQGNLTNDNSSSAKQDESATADS--PPLTAPINNNHANSQON-- : 451
cry5Aa tr : KQ--FKLPSURAGQYG--GLLQPYLWAEIVQDSVETRLYGQLPAVDPOAG-PNYV-SIDSSNPITQINHDUK-- : 441
cry5Ab tr : KQ--FKLPSURAGQYG--GLLQPYLWAEIVQDSVETRLYGQLPAVDPOAG-PNYV-SIDSSNPITQINHDUK-- : 441
cry5Ba tr : SR--KELQQLDIATYSTN--NSNNCHPYGLRLSYTDSRYDGDNDQDFTTNNNYC-HNSYTAFTLVNARHLNNA-K : 466
cry1Ac tr : G--YYUSGHQIMASPVGSGPEFTFPLYGTHGNAAPQORIVAOLGGGVYRTLS--TLYRRPFNIGNNQOLSVLDGCT-E : 387
cry1Ba tr : WSNTRHNTYRGHTIQSRPIGGGLNTSTHGATNTSINPVTLRFASRDVYRTSYAGVLLWGIYLEPFGPTVRFNFTNPONI : 422
cry1Ca tr : VG--RNFWUGGHRVISSLIGG--GNITSPITYGSEANQEV--PRSFTHNGPVFRTLSNPTRLRLLOQPWEAPPFNLRGVG-- : 388

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FIG. 3A

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00          *          520          *          540          *          560          *          580
axmi020 tr : -----ANYVGHNFQRAADKTAVPNGYIPNDN-----YTVPAHGKLGWISALHDELDNAN-N-----ADLVVSVW : 477
cry12Aa tr : -----QGYAFAQAPTGGWDESHFESDG-----APEGHKLNYIYTPSGDTLRDFIN-----VYTLISTP : 475
cry21Aa tr : --SQYLDLNSVHVNGG--QKVTGCSPISSNGN--SNNAAALPNQKINVIYSVQSNDRKPEK-----HADTYRRRGYHSSH : 526
cry21Ba1 t : --SQYDYSSINIDN--QGGGGCSAFPSYQ--SNNPILPNQKINVFYPGSSAHPIDPH-----TTDPDTWFKLGYSVSH : 520
cry5Aa tru : --TPQOGASGWNITNLHSGSVSGLSFIQORDGT--RLSAGHGCGFADTIYSLPATHYLSYLYGTPYQTSDNYSGHVGAIVGV : 518
cry5Ab tru : --TPQOGASGWNITNLHSGSVSGLSFIQORDGT--RLSAGHGCGFADTIYSLPATHYLSYLYGTPYQTSDNYSGHVGAIVGV : 518
cry5Ba tru : GSLQNVESLVVSTVNGGSGCICDAUINYLRPPTSKNESRPDQKINVLYPITETVKNKGTG-----G-----NLGVISAY : 536
cry1Ac tru : FAYGTSENLPASVYRKSGTYDSLDEHPQNN--NVPPRQGFSHRLSHVSHFRSGFSN-----SSVSILRAPM : 452
cry1Ba tru : SDRGTANYSQPYESPGLQKDSSETAPPETTE-----RPNYESYSHRLSHIGIILQSR-----VNVVPV : 480
cry1Ca tru : --VEFSTPTNSFTYRGRGTVDLSLETAPPEDN-----SVPPREGYSHRLCHATFVQRSG-----TPFLTITGVV : 448

          *          600          *          620          *          640          *          660
axmi020 tr : VKNDIFOENIIGSEKTVTTDDGTENRQCIIGIEADHHHTRS---TKRHELEFINGTNGSHLSSTND---CLYTTDNPV : 553
cry12Aa tr : TINELSTEKIKGFFAEKG-----YKKNQGIHYYGK-----PEIINGAQPUNJENQO---TLIFEFHASK : 533
cry21Aa tr : IPYDLVPENIIGDIDPDTKQP---SLIKGFFAEKGYGDS---IAYVSEPLNGANAVKLTESYQ---VLQHEVNTQ : 594
cry21Ba1 t : IPYDLTPCNVIGEIDQDTKQP---SLIKGFFAEKGYGGS---IAYVSEPLNGANAANKLTNQ---ILYHGVNLT : 588
cry5Aa tru : TPOEATLPNIIGQPDDEG---NVSTMGFFEFKASYG---GTVVKEMLNGANAHKLSPGQ---SIGPHITNV : 582
cry5Ab tru : TPOEATLPNIIGQPDDEG---NVSTMGFFEFKASYG---GTVVKEMLNGANAHKLSPGQ---SIGPHITNV : 582
cry5Ba tru : VPHELVPENIIGDVNADTKLP---LTKGKFFEFKASYG---GTVVKEMLNGANAHKLSNSQ---SVGICHTNQ : 608
cry1Ac tru : FSWIHSRAEFNNITASDS-----ITQIDAVKGNFLNG-SVISGPGGTGCDLVRLNSSGNINQNGYIEP : 523
cry1Ba tru : YSWIHSRAEADTNTICPNR-----ITQIDAVKASELPQGTIVVRGPGGTGCDILRENTTGGFG---PIRY : 548
cry1Ca tru : FSWIHSRAEADTNTICPNR-----ITQIDAVKASELPQGTIVVRGPGGTGCDILRENTTGGFG---PIRY : 516
          p K G 6

          *          680          *          700          *          720          *          740
axmi020 tr : -QKQRIYRVVITSAESLDIUIDGYKRGIT---TPNPNTSSSTSTQTKVVIQGLQGYOLINGPILDLTAGSHT : 622
cry12Aa tr : -AQTINIRMAST--QGTKCYFRIDNQEL-----QTNIPITSHNGYITG---NIGENYDLYTIGSYTITEGNT : 596
cry21Aa tr : -QKQRIYRVVITSAESLDIUIDGYKRGIT---TPNPNTSSSTSTQTKVVIQGLQGYOLINGPILDLTAGSHT : 666
cry21Ba1 t : -QKQRIYRVVITSAESLDIUIDGYKRGIT---TPNPNTSSSTSTQTKVVIQGLQGYOLINGPILDLTAGSHT : 659
cry5Aa tru : -GECICRBAASN--DNTNIFENVDTGGA-----NPFQOQINFASITVDN--NTGVQANGVYVVKSIATIDNSFT : 652
cry5Ab tru : -GECICRBAASN--DNTNIFENVDTGGA-----NPFQOQINFASITVDN--NTGVQANGVYVVKSIATIDNSFT : 653
cry5Ba tru : -QKQRIYRVVITSAESLDIUIDGYKRGIT---TPNPNTSSSTSTQTKVVIQGLQGYOLINGPILDLTAGSHT : 672
cry1Ac tru : STFRVVRVMASTVPIHLNNTGNSSIFS-----NTUPATATSLDNLQSSDFGYFESANFTSSLGNIVGRNFS : 593
cry1Ba tru : -QKQRIYRVVITSAESLDIUIDGYKRGIT---TPNPNTSSSTSTQTKVVIQGLQGYOLINGPILDLTAGSHT : 620
cry1Ca tru : -QKQRIYRVVITSAESLDIUIDGYKRGIT---TPNPNTSSSTSTQTKVVIQGLQGYOLINGPILDLTAGSHT : 592
          Y 6r RyA3

          *          760          *
axmi020 tr : -FGIALTATPSONVVIDRRETV-- : 644
cry12Aa tr : --HQIOHNDKNGHVLDRRETV-- : 618
cry21Aa tr : TILTONIN--SQDVLDRRETVSLP : 689
cry21Ba1 t : TILTONIN--SQDVLDRRETVSLP : 683
cry5Aa tru : TINVHLTNQGSSEVLDRRETV-- : 675
cry5Ab tru : TINVHLTNQGSSEVLDRRETV-- : 676
cry5Ba tru : SFYVHITNQGSSDLDRRETV-- : 695
cry1Ac tru : -----G-TACVVIDRRETV-- : 609
cry1Ba tru : -----G-NGEVIDRRETV-- : 636
cry1Ca tru : -PLFGAGSISSEGLRIDRRETV-- : 616
          6 6D4iEf6p

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FIG. 3B

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axmi021 : -HTONHSFSDNTSSSTGCVST-LESSLIPYVNVYATDQFNNSNVEDALKKLEKFSYSGDLTODAIDIFLG--DSGFDVLSLVNV : 79
cry12Aa tr : MATINEVYVNVYVNVLSDDAF-QQLDTTCFKSKYDEHAKAFKKKKKGAAGK-----DLLDVAUTYIT--TGEIDPLNVKKG : 79
cry21Aa tr : MTNPTILYPSYHNVLAHP IR-LDSFFDPFVETFKDLKGAEFEFGKTGYHDP-----LKQHLCHADTSQ--NGTVDYLALTKA : 75
cry21Ba1 t : MADLTLYPSYHNVLAHP IR-LDSIFDPFIDIFNALKGGWEEFAKTGYKDP-----LEQHLCHADNASQ--NGTIDYLALTKA : 75
cry5Aa tru : MAILNELYPSVYVNVLAYTP-PSFLPDAGTQATPADLTAYEQLLKNLEKGINAGTYSKAIADVLKGIFI--DDTINVQTYVNI : 80
cry5Ab tru : MAILNELYPSVYVNVLAYTP-PSFLPDAGTQATPADLTAYEQLLKNLEKGINAGTYSKAIADVLKGIFI--DDTINVQTYVNI : 80
cry5Ba tru : MATINELYVYVNVYVNVLAHP IK-EVDDPYVSNLLKGIQEGEEUWGTGQKKL-----FEDHLTHAUNLYK--TGKLDYFALTKA : 75
cry1Ac tru : -----HDNNPNINECIPYVCLSNPE-----VEVIGGERIE--TGVTPTIDISLSI : 42
cry1Ba tru : -----MTSN-RKNENEIINAVSNHSAQMDLLPDARIEDS-----LCIAEGNNIDPFVASTVCTGINT : 57
cry1Ca tru : -----MEEN-NQVQCIPYVCLSNPE-----VLIDGERIS--TGNSSIDISLSI : 41

axmi021 : IFSEHAGE-FIPYVVGALVPIINLLFGSES-----K--PDVSEONRAREALTHKELSAHDVQTLKAETHKELKDTGDLVQ : 149
cry12Aa tr : VLSVHT-LIPEVGTVASASTIVSTFMPKIFGDKPN--AKNIFEEELKPOEALIQODITNYODAINOKKFDLSIORTINLYT : 151
cry21Aa tr : SISEHG-LIPGADAVVPFNHMFVDFIFPKLFGSGSQNSQAQFELIIEKVKELVDEDFRNTITLNMILNLYDDEGTALSHPQ : 156
cry21Ba1 t : SISEHG-LIPDADAVVPFNHMFVDFIFPKLFGSGSQNSQAQFELIIEKVKELVDEDFRNTITLNLNDLDDGQTTLEHQP : 156
cry5Aa tru : GLSEHTL-AVPEIGIFTPTFGLFFAAANKHDAPPPPN--AKDIEAMKPAHQEHIIDRTLTADEQTFLLNGESGLONLAARYQ : 159
cry5Ab tru : GLSEHTL-AVPEIGIFTPTFGLFFAAANKHDAPPPPN--AKDIEAMKPAHQEHIIDRTLTADEQTFLLNGESGLONLAARYQ : 159
cry5Ba tru : SISEHG-FIPGAEAAVVPFNHMFVDFIFPKLFGANTEG-KDQQLNAINMDAANKVIVNKFSLYNLSTLNKTPELQGNLGLFQ : 155
cry1Ac tru : TQFVISE-FVPGAGFVLCGLDIDINGIFG-----PSQDAFLVQIEQLIQRIEEFARNQAISSRDELSNLYQIYA : 111
cry1Ba tru : AGRNIGVLGVDFAGQVASEYSLVGEIUPR-----GRDQIEIFLHVQELIQIETENARNTALARSQGLGDSFRANQ : 130
cry1Ca tru : VQFVSN-FVGGGGFLVGLIDFVVGIG-----PSQDAFLVQIEQLIQRIEIAEFARNAATANSGLGNFNINLV : 110

axmi021 : KDNNAVAGRTNGETPPSFDSN-----TDALKAEIRSQITATNLFVQRIEQAIEGY-----EENLPLPHHT : 210
cry12Aa tr : VADNNNDYVTAK-----TQENLNSILTSIDISIFIEGYETGG-----LPIYHA : 194
cry21Aa tr : NDVQIAICQGGQPGGLH-LDQTPACTPT--TDHLISVRESFEKADRTIETALIEFKNPHLSTNDNTPDFNSDTVLTLPHYT : 235
cry21Ba1 t : NDVQIAICQGGQPGGLI-LDEKHPPCTPT--KNHLSVRESFEKADRTIETALIEFKNPHLSTNDNTPDFNSDTVLTLPHYT : 233
cry5Aa tru : STDDIQSHGG-----FNKVDGLIKKFFDEVLSNSFYTDRLP--VFITDNTADRTLTGLGYHA : 217
cry5Ab tru : STDDIQSHGG-----FNKVDGLIKKFFDEVLSNSFYTDRLP--VFITDNTADRTLTGLGYHA : 217
cry5Ba tru : NADVOAICQGSTPERVNFQDCTPCNPQPCDDLDVASFEDTANQFTQHLIEFKNPHLSTNDNTPDFNSDTVLTLPHYT : 237
cry1Ac tru : ESFREWADPTNP-----ALREERIQFNDNMSALTATLFAVQNYQVP-----LUSVYV : 162
cry1Ba tru : QSEEDWLENRRDAR-----TRSVLYTQYIALELDFLNAPLEFAIRNQ-----VPLHUYHA : 181
cry1Ca tru : EAFKEWEEDPMNP-----ATRTRVIDRILRGLDGLERDLSERISGFVFP-----LUSVYA : 161

axmi021 : IADSHHIFPLKDVCEHGAELGIAN-----TTLINYQGGQDCHREHNSKAYSHRNIGLQAKN-NGNNNNNNNNNNNNNNNNNN : 288
cry12Aa tr : HVNNAHIFPLKDVCEHGAELGIAN-----SDKEVPTHKKYVKNTHHNVHAEVAKAFNGSDKFKS-LDVNSYNNKANNYIFGHENV : 272
cry21Aa tr : TGAITNIFHOGYLOFAERFKS-VNYDESFINQTVVDFORRHODFSTTSTTEKFKPTLNP-SNKESVNNYNNYVRSHTLOS : 316
cry21Ba1 t : TATNIFHOGYLOFAERFKS-VDYDEAFINQTVVDFORRHODFSTTSTTEKFKPTLS--NNKSEINTYNNKVESHTLNC : 313
cry5Aa tru : ILSEHTLILRLDITTKGPTDSDKINFTPDAISFKTDKNNKILKSKTLYDVQKQASGYTPSDLESFAKKOKIETITHC : 300
cry5Ab tru : ILSEHTLILRLDITTKGPTDSDKINFTPDAISFKTDKNNKILKSKTLYDVQKQASGYTPSDLESFAKKOKIETITHC : 300
cry5Ba tru : TVNIFHOGYLOFAERFKS-VNYDESFINQTVVDFORRHODFSTTSTTEKFKPTLNP-SNKESVNNYNNYVRSHTLOS : 316
cry1Ac tru : QANVHIFPLKDVCEHGAELGIAN-----ATLSRYNDETRLLGNFYDVAVRWYNTGERVUG-PDSRDVVRVYNGRRLDILGV : 240
cry1Ba tru : QANVHIFPLKDVCEHGAELGIAN-----QELQRYREROVERTDSDYCVVWYNTGNSLRG-TNAAVVRVYNGRRLDILGV : 259
cry1Ca tru : QANVHIFPLKDVCEHGAELGIAN-----NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 239

axmi021 : LDTVILQURILKVTIEL-----DITLQOTRGIFSDLS--GR-----GGTQSNRYDYDAVQG-----YAPPFVG--FDTK : 349
cry12Aa tr : LDELVLUPFTDPDHYOK-----EVEIEFTRISSPIYQVPKPN--HONTSSSIMPDLFHYQGLVLEKFSRTDNDGLAKI : 347
cry21Aa tr : LDTAATUPTLNNVNVES-NV-DIQDQTRLVFSVDAVQWEGN--DNITSNIDVLTPINT-----GIGQES-SDLRKF : 385
cry21Ba1 t : LDTAATUPTLNNVNVES-NV-DIQDQTRLVFSVDAVQWEGN--DDISYTRRSINHYSK-----GDTPGDVNSAQSLS : 383
cry5Aa tru : LDPARLPFTFPDLVETGSG-DISLOKTRILSPFIPIRTAD--GLTLNNTSDTENVNPN-----YENGNGAFPNPKER : 371
cry5Ab tru : LDPARLPFTFPDLVETGSG-DISLOKTRILSPFIPIRTAD--GLTLNNTSDTENVNPN-----YENGNGAFPNPKER : 371
cry5Ba tru : LDTAATUPTLNNVNVES-NV-DIQDQTRLVFSVDAVQWEGN--DNITSNIDVLTPINT-----GIGQES-SDLRKF : 385
cry1Ac tru : LDELVLUPFTDPDHYOK-----EVEIEFTRISSPIYQVPKPN--HONTSSSIMPDLFHYQGLVLEKFSRTDNDGLAKI : 347
cry1Ba tru : LDELVLUPFTDPDHYOK-----EVEIEFTRISSPIYQVPKPN--HONTSSSIMPDLFHYQGLVLEKFSRTDNDGLAKI : 347
cry1Ca tru : LDTAATUPTLNNVNVES-NV-DIQDQTRLVFSVDAVQWEGN--DNITSNIDVLTPINT-----GIGQES-SDLRKF : 385

axmi021 : LNVV-----NDFGYKDLTAIQTFT--GDRIDSIUQSFKYNSGEFF-LTNLGNKPGNNPVIPN-----SRDNGTISAKGS : 416
cry12Aa tr : FTGI-----RNTYKSPNTHETVH--VDFSYNTQSSGNYSRGSNN-PIPIDLNNPIISTCIRNSFYK-----AIAGSSVLVNFK : 418
cry21Aa tr : TYPR-----IELQSMQFHGQYVNS--KSVEHCYSDGLKLYNNKKT--ITAGVSNIDESNQNKKHNYGP-----VINSPTDIDVNV : 456
cry21Ba1 t : RYPR-----LELSKVQFYTHDQRS--NGVRHCYTSGFNITFNDNS-SMSAKQDESATADS--P-----PLTAPKNNAN : 448
cry5Aa tru : ILKQ-----FKLYPSURAGQYG--GLLOPYLWAIENQDSVET-RYVQGLPAVDPOAG-PNYVSI-----DSSNPTIQIYHD : 438
cry5Ab tru : ILKQ-----FKLYPSURAGQYG--GLLOPYLWAIENQDSVET-RYVQGLPAVDPOAG-PNYVSI-----DSSNPTIQIYHD : 438
cry5Ba tru : VYSR-----KEQQLDIATYSTN--NSNNCHPYGLRLSYTDGS-RYVYGDNPQDFTTSNNNYCHN-----SYTADITLVNAR : 460
cry1Ac tru : HRG-----YYVUSGQINASPVGFSGEPTFFLYGTNGNAAPQORTVAQLCGQVYRTLSSTLYRR--PFIENNNQOLS : 381
cry1Ba tru : SRWENRHHMTURGHITQSRP--IGGGLNTSTHGATNTSINVTIRFASRDVYRTESYAGVLLUGIYLEPIHGVITVRPFT : 417
cry1Ca tru : FSVG--RNFFVGGHRVSSLI--GGGNITSPYIGREANGPEP-RSFTFNGPVFRTLSNPTLRLL-----QOPWAPPPLRL : 364

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FIG. 4A

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      00          *      520          *      540          *      560          *      580
axmi021 : RPS-----ANYVGHNFQRANKTIVVSNQYVIPNDN-----YTVPAHGKLGWISALHDELNDAN-N-----ADLVVS : 475
cry12Aa tr : DGT-----QGYAFQAAPTGGAWDHSHFIESDG-----APEGHKLNVLYTSPGDTLRDFIN-----VY : 469
cry21Aa tr : SQN-----SQYLDLNSVHVNGG-QKVITGCSPISSNGN-----SNNAAALPNQKINVIYSVQSNDKPEK-----HADTYRKW : 520
cry21Ba1 t : SQN-----SQYDYSSINIDN--QGGGGCSAFPSTYQ-----SNNPILPNQKINVIYFYGSSAHPIDPH-----TTDPDTDFKL : 514
cry5Aa tru : TWK-----TPQGASGUNTNLHRSVSGLSFIFQORDGT-----RLSAGHGCGFADTIYSLPATHYLSYLYGTPYQTSNDNYSGHVG : 512
cry5Ab tru : TWK-----TPQGASGUNTNLHRSVSGLSFIFQORDGT-----RLSAGHGCGFADTIYSLPATHYLSYLYGTPYQTSNDNYSGHVG : 512
cry5Ba tru : HLYNAKGSLONVESLVVSTVNGGSGSCICDAVINYLRRPQTSKNESRPDQKINVLPIITETVKNKGTG-----G-----NL : 530
cry1Ac tru : VLDGTEFAYGTSSNLPSAVYRKSGTWDSDLETPPONN-----NVPPRQCFSHRLSHVSHFRSGFSN-----SS : 444
cry1Ba tru : NPQN-ISDRGTANYSQPYESPGLQDKDSETEPPPETTE-----RPNYESYSHRLSHIGIILQSR----- : 475
cry1Ca tru : GVEG---VEFSTPTNSFTYRGRGTWDSLTEPPPEDN-----SVPPREGYSHRLCHATFVQRSG-----T : 440

      *      600          *      620          *      640          *      660
axmi021 : VMVKNDIFQENIIGSIKTVTTDDGTTENRQOIGIADKHMTRS---TKRHELEINCTNGSMLSLSSTND---QYYTINPI : 551
cry12Aa tr : TLSTPTINELSTEKIKGFPAEKG-----YMKNOGIMKYGK-----PEEINGCAQPVNLENQO---TIFEFHAS : 531
cry21Aa tr : GYSSSHIPYDLVPENVIGDIDPDTKOPS-LLKGFPAEKGYGDS---IAYVSEPLNCANAVKLTSYQ---VLCHEVTNQ : 592
cry21Ba1 t : GYSSSHIPYDLTPONVIGEIDODTKOPS-LLKGFPAEKGYGGS---IEYVSEPLNCANAAKLTNLQ---ILYVQYTNL : 586
cry5Aa tru : ALMGVSTPQEAATLPNTIGQPDEQG---N-VSTHGPFERASYG---GTVVKEPLNCANAKKLSFGQ---SHGPHITNV : 580
cry5Ab tru : ALMGVSTPQEAATLPNTIGQPDEQG---N-VSTHGPFERASYG---GTVVKEPLNCANAKKLSFGQ---SHGPHITNV : 580
cry5Ba tru : GVHSAYVPHLVPENVIGDVNADTKLPL-TQKGFPAEKGSEYNNRGISLVRLEINCNNAVKLSNSQ---SVGQYTNQ : 606
cry1Ac tru : VSHIRAPMFSWIHRSAEFNNIIAS-----DSHTQIDAVGNFLFNG-SVISGPGSTGCDLVRNLSSGNNIQRGYEUPPHFP : 521
cry1Ba tru : --VVVPM-YSWIHRSAEADRTNTIGP---NRHTQIDAVGNFLFNG-SVISGPGSTGCDLVRNLSSGNNIQRGYEUPPHFP : 521
cry1Ca tru : PFTTGYVVSUTHRSAATLTNTIDP---ERNQIDAVGNFLFNG-SVISGPGSTGCDLVRNLSSGNNIQRGYEUPPHFP : 514
      6          p          K          G          6

      *      680          *      700          *      720          *      740
axmi021 : VVQRQQLYRVATTSAESLDHVIDGYKRG---TPHPNTSSTSTOTOKVIIQGLQGYQLINGPTLIDLTAGSHT--- : 622
cry12Aa tr : KA-AQMTLIRAST--QGTGGERLDNQEL---QTNIPTSNHYVTG---NIGENYDLYTGESYITIEGNHT--- : 596
cry21Aa tr : TQ-OKVRIIRATGGDTAASVDEHIGPSG---NDNTNEGHNFSSVSSRNKMFVQGNNGKYVLNLTDSIELPS--G- : 664
cry21Ba1 t : TQ-OKVRIIRATKNDTTASVDEHIGPNN---QDINHSPDIPPRSN-NKHVFQENGKYLVDITLVDSELPS--G- : 657
cry5Aa tru : TS-GEVQICRNASN---DNTNFFPNVDTGGA---NPIFQOINFASVMDN---NTGVQGANGVYVVKSIATIDNSFTVVKIP : 650
cry5Ab tru : TS-GEVQICRNASN---DNTNFFPNVDTGGA---NPIFQOINFASVMDN---NTGVQGANGVYVVKSIATIDNSFTVVKIP : 651
cry5Ba tru : TK-OKVRIIRASKG---DNNQYFNVDLSE---NPFRRNSISFGSTESS--VVGQGANGVYVVKSIATIDNSFTVVKIP : 672
cry1Ac tru : SSTRVRYVVRASVTPFIHLMNNIGNSSIFS---NTUPATATSLDNLQSSDFGYFESANAFSTSSLGNIVGVNRVFG--- : 594
cry1Ba tru : LI-ORVRIIRASTVDFDFFSRGGTTVMN---FFFLRTMNSGDELKYGNFVRRAFITPFTFTQIQDIIRTSIQGLS--- : 620
cry1Ca tru : LI-ORVRIIRASTVDFDFFSRGGTTVMN---FFFLRTMNSGDELKYGNFVRRAFITPFTFTQIQDIIRTSIQGLS--- : 592
      Y 6r Rya3

      *      760          *      780          *      800          *      820
axmi021 : ---FGIMLTANASQNVHIDRHEEVEIATTEPVTIPNTPIKTYTNPPNPQOVLWTAQPGILGDIVNYHINLYNHL- : 693
cry12Aa tr : ---LQIQHNDKNGVLDREVEVEKD----- : 618
cry21Aa tr : QQTILFQNTN--SODLELDREVEVEISLP----- : 689
cry21Ba1 t : QLTILFQNTN--DODLELDREVEVEIP----- : 683
cry5Aa tru : AKTINVHLINQGSSEVLDREVEVE----- : 675
cry5Ab tru : AKTINVHLINQGSSEVLDREVEVE----- : 676
cry5Ba tru : --SFVYHITNQGSSDLVDREVEVE----- : 695
cry1Ac tru : ---TAGVHIDRHEEVEIIVT----- : 609
cry1Ba tru : ---NGGEVHIDRHEEVEIIVT----- : 636
cry1Ca tru : -PLFGAGSIS--SGELHIDRHEEVEIIVT----- : 616
      6 6D4IEf6p

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FIG. 4B

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